PB81-152522

Effects of Halogenated Aromatic Compounds on the Metabolism of Foreign Organic Compounds

Purdue Univ.
Lafayette, IN

Prepared for

Health Effects Research Lab.
Cincinnati, OH

Jan 81

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-81-010		PB81-152522

4. TITLE AND SUBTITLE	5. REPORT DATE
Effects of Halogenated Aromatic Compounds on the Metabolism of Foreign Organic Compounds.	January 1981 Issuing Date.
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Gary P. Carlson	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
School of Pharmacy and Pharamcal Sciences Department of Pharmacology and Toxicology Purdue University West Lafayette, IN 479-7	11. CONTRACT/GRANT NO. R805070

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. EPA, HERL Toxicological Assessment Branch Cincinnati, Ohio 45268	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

. This work was initiated to extend our previous findings on the induction of xenobiotic metabolism by the halogenated benzenes. Particular interest was focused on studying further the relationship between their long-term induction and their storage in body fat. A second objective was to determine if the brominated benzenes caused hepatic porphyria similar to that observed with the fungicide hexachlorobenzene. A third aim was to extend our observations to other halogenated benzenes in the enhancement of esteratic pathways of xenobiotic metabolism.

In contrast to hexachlorobenzene, the brominated benzenes, including the fully substituted hexabromobenzene, did not induce hepatic prophyria to any significant degree and did not cause increases in the excretion of prophyrins.

Studies on the distribution and elimination of 1,2,4-trichlorobenzene and 1,2,4-tribromobenzene indicated that their prolonged inductive effects are related to their storage and slow release from body stores, particularly adipose tissue. The administration of Aroclor 1254 resulted in prolonged increases in xenobiotic metabolism which could also be enhanced by starvation.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Halogenated Aromatics Rats	Xenobiotic Metabolism Induction Storage Porphyria Starvation	

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA 600/1-81-010
January 1981

PB81-152522

EFFECTS OF HALOGENATED AROMATIC COMPOUNDS ON THE METABOLISM OF FOREIGN ORGANIC COMPOUNDS

by

Gary P. Carlson
School of Pharmacy and Pharmacal Sciences
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN 47907

Grant No. R805070

Project Officer

Merrel Robinson
Toxicological Assessment Branch
Health Effects Research Laboratory
Cincinnati, OH 45268

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO, 45268

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN: EPA FORM 2471)
TYPING GUIDE SHEET

FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

In this report the results are presented from a three year study on the effects of halogenated aromatic hydrocarbons on the metabolism of foreign organic compounds. This work was initiated to extend our previous findings on the induction of xenobiotic metabolism by halogenated benzenes and to extend our observations to other halogenated aromatic compounds.

R.J. Garner
Director
Health Effects Research Laboratory

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD. BEGIN SECTIONS HERE

ABSTRACT

This work was initiated to extend our previous findings on the induction of xenobiotic metabolism by the halogenated benzenes. Particular interest was focused on studying further the relationship between their long-term induction and their storage in body fat. A second objective was to determine if the brominated benzenes caused hepatic porphyria similar to that observed with fungicide hexachlorobenzene. A third aim was to extend our observations to other halogenated aromatic compounds. The final objective was to examine the role of the halogenated benzenes in the enhancement of esteratic pathways of xenobiotic metabolism.

In contrast to hexachlorobenzene, the brominated benzenes, including the fully substituted hexabromobenzene, did not induce hepatic porphyria to any significant degree and did not cause increases in the excretion of porphyrins. This would suggest that they do not present a health hazard in this manner.

A number of trichlorophenols were assessed for their potential to increase xenobiotic metabolism. However, they did not increase any of the indicators of induction. In vitro they were found to be inhibitors of both mixed function oxidase and conjugative enzymes.

The brominated diphenyl ethers, commercial mixtures used as fire retardants, were found to be potent inducers of xenobiotic metabolism. Administration of low doses for 90 days resulted in effects which were prolonged far beyond the period of administration. The chlorinated diphenyl ether isomers did not share the potent inducing ability of the brominated diphenyl ethers. Nevertheless, definite structure activity relationships were found.

The chlorinated benzo-p-quinones examined were not found to be inducers. However, they did demonstrate a very strong cumulative toxicity. It was shown that this was not due to liver damage or to methemoglobinemia. The chlorinated dibenzofurans were identified as potent inducers of the 3-methylcholanthrene-type by their high induction of benzo(a)pyrene hydroxylase activity and shift in absorption of cytochrome P-450 toward 448 nm.

Studies on the distribution and elimination of 1,2,4-trichlorobenzene and 1,2,4-tribromobenzene indicated that their prolonged inductive effects are related to their storage and slow release from body stores, particularly adipose tissue. This relationship can be altered by starvation or administration of phenobarbital to rapidly mobilize the stored material. The administration of Aroclor 1254 resulted in prolonged increases in xenobiotic metabolism which could also be enhanced by starvation.

The halogenated benzenes induced esterases associated with the metabolism of acetanilide, procaine, and phenyl acetate. The structure activity

BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
EPA Form 2350-4 (4-80)
(PREVIOUSLY CID. EPA FORM 2971)
PAGE NUMBER
U.S. GOVERNMENT PRINTING OFFICE: 1980-060-639
TYPING GUIDE SHEET

relationships among the isomers were similar to those observed for the mixed function oxidase reactions. They were also able to protect against the lethality and inhibition of cholinesterase activity associated with the organophosphate insecticides parathion and malathion and their active metabolites, paraoxon and malaoxon, by enhancing the metabolic detoxification of these compounds via both esterases and mixed function oxidases.

This report was submitted in fulfillment of Grant No. R805070 by the Purdue Research Foundation under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period from March 24, 1977 to September 15, 1980, and work was completed as of September 23, 1980.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
EPA Form 2350-4 (4-80)
PAGE NUMBER
(PREVIOUSLY CIM EPA FORM 2[illegible])
TYPING GUIDE SHEET

CONTENTS

Foreword iii
Abstract iv
Figures ix
Tables x
Abbreviations xiii
Acknowledgment xiv

1. Introduction 1
   - Hepatic Porphyria 1
   - Halogenated Aromatics as Inducers of Xenobiotic Metabolism . 1
   - Relationship between Induction and Storage 2
   - Esterase Activity 3
2. Conclusions 4
   - Hepatic Porphyria 4
   - Halogenated Aromatics 4
   - Relationship between Induction and Storage 5
   - Esterase Activity 6
3. Recommendations 7
4. Materials and Methods 9
   - Hepatic Porphyria 9
   - Halogenated Aromatics 10
     - Chlorinated Phenols 10
     - Brominated Diphenyl Ethers 10
     - Chlorinated Diphenyl Ethers 11
     - Benzoquinones 12
     - Dibenzofurans 12
   - Relationship between Induction and Storage 12
     - Duration of Induction by PCB and Influence of Starvation 13
   - Esterases 13
     - Arylesterase 13
     - Acetanilide and Procaine Esterases 13
     - Esterases and Pesticide Toxicity 14
5. Results 15
   - Hepatic Porphyria 15
   - Halogenated Aromatics 16
     - Chlorinated Phenols 16
     - Brominated Diphenyl Ethers 16
     - Chlorinated Diphenyl Ethers 17
     - Benzoquinones 18
     - Dibenzofurans 18

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN, EPA FORM 2871)
☆ U.S. GOVERNMENT PRINTING OFFICE 1980-600-639
vii
TYPING GUIDE SHEET

Relationship between Storage and Induction 18
Halogenated Benzenes 18
Duration of Induction by PCB and Influence of Starvation 20
Esterase Activity 20
Acetanilide and Procaine Esterases 20
Esterases and Pesticide Toxicity 21
Arylesterase 22

References 66

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN, EPA FORM 287)
TYPING GUIDE SHEET

FIGURES

Number		Page
1	Effect of 10 µmols/kg/day of chlorinated diphenyl ethers for 3 days po on EPN detoxification	23
2	Effect of 10 µmols/kg/day of chlorinated diphenyl ethers for 3 days po on aryl hydrocarbon hydroxylase	24
3	Effect of 10 µmols/kg/day of chlorinated diphenyl ethers for 3 days po on NADPH cytochrome c reductase	25
4	Effect of 10 µmols/kg/day of chlorinated diphenyl ethers for 3 days po on cytochrome P-450	26
5	Decline with time in rats receiving TBB (1 mmol/kg/day) for 7 days po	27
6	Decline with time in rats receiving TCB	28
7	Urinary and fecal excretion of ^{14}C during and after 7 days of administration of [^{14}C]TBB	29
8	Urinary and fecal excretion of ^{14}C during and after 7 days of administration of [^{14}C]TCB	30
9	Effect of phenobarbital treatment on EPN detoxification, p-nitroanisole demethylation, and cytochrome P-450 in livers from rats receiving TBB	31
10	Effect of phenobarbital treatment on EPN detoxification, p-nitroanisole demethylation, and cytochrome P-450 in livers from rats receiving TCB	32

TABLES

Number		Page
1	Effect of hexabromobenzene po on porphyrin production and excretion in female rats	33
2	Effect of 1,4,-dibromobenzene po on porphyrin production and excretion in female rats	34
3	Effect of 1,2,4-tribromobenzene po on porphyrin production and excretion in female rats	35
4	Effect of trichlorophenols on EPN detoxification, cytochrome c reductase and cytochrome P-450	36
5	Effect of trichlorophenols in vitro on p-nitroanisole O-demethylation and EPN detoxification	37
6	Effect of trichlorophenols on glucuronyltransferase	37
7	Effect of trichlorophenols in vitro on glucuronyltransferase	38
8	Hepatotoxicity of the trichlorophenols	38
9	Composition of bromodiphenyl ethers	39
10	Effect of bromodiphenyl ethers on liver wt./body wt., NADPH cytochrome c reductase, and cytochrome P-450	39
11	Effect of bromodiphenyl ethers on EPN detoxification, p-nitroanisole demethylation, benzo[a]pyrene hydroxylase, and UDP-glucuronyltransferase	40
12	Effect of pentabromodiphenyl ether or octabromodiphenyl ether on EPN detoxification, p-nitroanisole demethylation, cytochrome c reductase activity, and cytochrome P-450	41
13	Effect of pentabromodiphenyl ether on EPN detoxification, p-nitroanisole demethylation, NADPH cytochrome c reductase, and cytochrome P-450	42
14	Effect of octabromodiphenyl ether on EPN detoxification, p-nitroanisole demethylation, NADPH cytochrome c reductase, and cytochrome P-450	43

15 Comparison of oral versus intraperitoneal routes of administration on 2,5-dichloro-p-benzoquinone 44

16 Effect of benzoquinone and derivatives on methemoglobin formation and sorbitol dehydrogenase 45

17 Effect of benzoquinones on the microsomal system for metabolism of foreign organic compounds 46

18 Effect of dibenzofuran, 2,8-dichlorodibenzofuran, and octachlorodibenzofuran on EPN detoxification, benzpyrene hydroxylase, NADPH-cytochrome c reductase, and cytochrome P-450 47

19 Content of ^{14}C-labeled 1,2,4-tribromobenzene and 1,2,4-trichlorobenzene in tissues 48

20 Influence of starvation on EPN detoxification, p-nitroanisole demethylation, and cytochrome P-450 content of liver in rats treated with 1,2,4-tribromobenzene 49

21 Influence of starvation on EPN detoxification, p-nitroanisole demethylation, and cytochrome P-450 content of liver in rats treated with 1,2,4-trichlorobenzene 49

22 Influence of starvation on content of ^{14}C in abdominal fat and liver after TCB or TBB 50

23 Influence of starvation on urinary excretion of ^{14}C after TCB or TBB administration 50

24 Effect of Aroclor 1254 on EPN detoxification and p-nitroanisole demethylation 51

25 Effect of Aroclor 1254 on NADPH cytochrome c reductase and cytochrome P-450 52

26 Effect of halogenated benzenes on acetanilide hydroxylase and acetanilide esterase 53

27 Effect of halogenated benzenes on procaine esterase 53

28 Effect of brominated and chlorinated benzenes on the lethality of malathion 54

29 Effect of 1,2,4-trichlorobenzene and 1,2,4-tribromobenzene on the lethality of malaoxon 55

30 Effect of brominated and chlorinated benzenes on the lethality of parathion 56

31 Effect of brominated and chlorinated benzenes on the lethality of paraoxon 57

32 Effect of 1,2,4-trichlorobenzene on cholinesterase activity in the brain, liver, plasma, and red blood cells of mice challenged with malathion 58

33 Effect of 1,2,4-tribromobenzene on cholinesterase activity in the brain, liver, plasma, and red blood cells of mice challenged with malathion 59

34 Effect of brominated and chlorinated benzenes on malathion carboxylesterase 60

35 Effect of brominated and chlorinated benzenes on malaoxon carboxylesterase 61

36 Effect of 1,2,4-trichlorobenzene on cholinesterase activity in the brain, liver, plasma, and red blood cells of mice challenged with paraoxon 62

37 Effect of 1,2,4-tribromobenzene on cholinesterase activity in the brain, liver, plasma, and red blood cells of mice challenged with paraoxon 63

38 Effect of brominated and chlorinated benzenes on paraoxon dearylation by mouse plasma and liver 64

39 Effect of halogenated benzenes on arylesterase 65

LIST OF ABBREVIATIONS

ABBREVIATIONS

AHH	-- aryl hydrocarbon hydroxylase
ALA	-- delta-aminolevulinic acid
Ci	-- Curie
DBB	-- dibromobenzene
DCB	-- dichlorobenzene
EDTA	-- ethylenediaminetetraacetic acid
EPN	-- O-ethyl O-p-nitrophenyl phenylphosphonothioate
g	-- gram or gravity
HBB	-- hexabromobenzene
HCB	-- hexachlorobenzene
ip	-- intraperitoneal
kg	-- kilogram
LD50	-- calculated dose which will kill 50% of a group of animals
mg	-- milligram
NADPH	-- nicotinamide-adenine dinucleotide phosphate, reduced
nm	-- nanometers
PBG	-- porphobilinogen
PCB	-- polychlorinated biphenyl
po	-- per os
S.E.	-- standard error
TBB	-- tribromobenzene
TCB	-- trichlorobenzene
Tris	-- tris(hydroxylmethyl)aminomethane

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 281)
PAGE NUMBER
U.S. GOVERNMENT PRINTING OFFICE
xiii
TYPING GUIDE SHEET

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

ACKNOWLEDGMENTS

The very able collaboration, technical assistance and encouragement of the following individuals is gratefully appreciated: Dr. Charles Henrikson, Mrs. Kathryn Johnson, Mr. Eric Smith, Mrs. Judie Smith, Mrs. Shelley Steva, and Miss Beverley Townsend of Purdue University and Dr. Nelson Fausto of Brown University. The author is grateful for the help of Mrs. Ruth Hershberger and Mrs. Bruce McDaniel in the preparation of the final report.

The following material was reprinted from copyright articles. Some text material and Tables 1 through 3 are reproduced from Experientia, Vol. 35, pages 513-514, copyright 1979 by Birkhauser Verlag, Basel, Switzerland. Some text material and Figures 1 through 4 are reproduced from Drug and Chemical Toxicology in press in 1980 and copyright by Marcel Dekker, New York. Some text material and Tables 4 through 8 are reproduced from Toxicology, Vol. 11, pages 145-151, copyright 1978 by Elsevier/North Holland Scientific Publishers, Ltd., Limerick, Ireland. Some text material and Tables 9 through 11 are reproduced from Toxicology Letters, Vol. 5, pages 19-25, and Tables 12 through 14 from Vol. 6, pages 207-212, both copyright 1980 by Elsevier/North Holland Biomedical Press, Amsterdam. Some text material and Tables 19 through 23 and Figures 5 through 10 are reproduced from the Journal of Toxicology and Environmental Health, Vol. 6, pages 737-749, copyright 1980 by Hemisphere Publishing Corp., Washington, D.C. Some text material and Tables 24 and 25 are reproduced from Life Sciences, Vol. 27, pages 1571-1576, copyright 1980 by Pergamon Press, Ltd., New York. Some text material and Tables 26 and 27 are reproduced from Research Communications in Chemical Pathology and Pharmacology, Vol. 25, pages 181-184, copyright 1979 and Vol. 30, pages 361-364, copyright 1980 by PJD Publications, Ltd., Westbury, New York.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3.5"
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN: EPA FORM 2ET)
xiv
TYPING GUIDE SHEET

SECTION 1

INTRODUCTION

There is great interest in induction of xenobiotic metabolism by halogenated aromatics and resultant interactions of these compounds with the actions of concomitantly administered chemicals or naturally occurring chemicals, such as hormones. Most studies, however, have focused on the polychlorinated biphenyls, polybrominated biphenyls, and chlorinated dibenzo-p-dioxins. Previous studies in our laboratory have indicated that the chlorinated and brominated benzenes are also inducers of xenobiotic metabolism.

There are definite structure-activity relationships, with brominated benzenes more active than chlorinated analogs. In general, the greater the degree of halogenation, the greater the degree of induction. Second, effects are prolonged beyond the period of administration of the halogenated benzenes.

These observations on the actions of halogenated benzenes on mixed function oxidases led to the necessity of extending the investigation to actions on porphyria and esterase activity, to examine the relationship between storage of halogenated benzenes and their long-term effects, and to explore the possibility that other halogenated aromatic compounds might also be inducers of xenobiotic metabolism.

Hepatic Porphyria

The fungicide hexachlorobenzene has been observed in a number of laboratory species and in humans to cause more porphyrinogenic effects than do the less chlorinated benzenes. Brominated compounds are often more active than chlorinated analogs, so it appeared important to investigate effects of the fire retardant hexabromobenzene (HBB) on porphyrin synthesis. Male rats feeding on HBB at levels up to 160 ppm for 12 weeks did not result in porphyria, although there was an insignificant tendency toward increased liver porphyrins. In the present studies, the effects of two less halogenated benzenes, 1,4-dibromobenzene and 1,2,4-tribromobenzene, were examined. They had been found more potent than the chlorinated analogs in induction of xenobiotic metabolism.

Halogenated Aromatics as Inducers of Xenobiotic Metabolism

Chlorinated benzenes are known to induce microsomal enzymes associated with xenobiotic metabolism, but very little is known of the potential of chlorinated phenols. The trichlorophenols used industrially in synthesis and formed by over-chlorination of water containing phenols and by microbial

transformation of chlorobenzenes are of special interest. It appeared possible that the chlorinated benzenes were not direct inducers of xenobiotic metabolism, but only upon conversion to chlorinated phenols. This study was undertaken to determine whether or not trichlorophenols could alter xenobiotic metabolism.

Brominated diphenyl ethers are structurally similar to several halogenated aromatic compounds known to induce xenobiotic metabolism. They are commercial mixtures, so it was important to compare them with diphenyl ether itself, bis(p-bromodiphenyl)ether, and the fully brominated diphenyl ether. They are used as fire retardants, but little has been studied about their toxicity.

Chlorinated diphenyl ethers are contaminants of chlorinated phenols and readily concentrated, for example, by fish. Because of the structural-activity relationships among chlorinated biphenyls and chlorinated dioxins, it was of interest to examine the chlorinated diphenyl ethers and compare the potency of individual isomers to commercial mixtures of brominated diphenyl ethers.

Benzoquinones were examined for effects as inducers, and although they did not qualify in this respect, it was found that they have very cumulative toxicity. Benzofurans were found to be very potent inducers of xenobiotic metabolism.

Relationship between Induction and Storage

Municipal wastewaters contain several chlorinated and brominated benzenes which induce xenobiotic metabolism at low dose levels. It is known that brominated isomers are generally more potent inducers of hepatic microsomal enzymes than chlorinated isomers and that the 1,2,4-trihalogenated compound is a potent inducer compared to other isomers.

Studies with 1,2,4-trichlorobenzene and 1,2,4-tribromobenzene show they could cause enzyme induction, even at low doses. Observations suggest that storage in body tissues, with slow release after dose termination, prolongs induction of xenobiotic metabolism. Storage problems of such compounds and their release by starvation may be important with these highly lipid-soluble compounds in humans or animals subjected to altered food intake, as in starvation dieting or food unavailability.

This study correlated body burdens of these compounds with sustained induction of xenobiotic metabolism. The influence that starvation or phenobarbital treatment may have in conjunction with administration of these two halogenated benzenes was observed. High doses of the compounds were given over a short period to shorten the time needed to attain high levels of the halogenated benzenes in fat, rather than low doses for prolonged periods, as done previously.

Numerous studies have shown the ability of the polychlorinated biphenyls to induce xenobiotic metabolism. In view of the results with the halogenated benzenes, similar experiments were conducted to determine the length of time

the effects of PCBs on xenobiotic metabolism could be observed. Whether or not starvation might have an effect on storage and release, and subsequently the inducing ability of these compounds, was also investigated.

Esterase Activity

Little is known about the ability of halogenated and chlorinated benzenes to alter esterases, except that over 200 pharmacologically active compounds are esters. Hexabromobenzene is compared in this study with less halogenated benzenes in regard to metabolism of acetanilide, which is biotransformed by both an oxidative reaction and an esterase. Hydrolysis of procaine was also studied.

Cholinesterase activity in brain, liver, plasma, and red blood cells was studied in mice to determine effects of alterations in the metabolism of malathion and paraoxon by the halogenated benzenes on the anticholinesterase activity of these pesticides. Carboxylesterase activity in liver and plasma of mice was measured spectrophotometrically to determine effects of the halogenated benzenes on metabolism of malathion and malaoxon. A similar method was used for paraoxon dearylation to p-nitrophenol.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN: EPA FORM 287)
TYPING GUIDE SHEET

SECTION 2

CONCLUSIONS

This study increased in scope and depth as research progressed. It became apparent that interactions affecting xenobiotic metabolism were more complex than originally hypothesized. The more obvious conclusions are stated under subheadings for hepatic porphyria, halogenated aromatics, relationship between induction and storage, and esterase activity.

Hepatic Porphyria

The brominated benzenes did not greatly increase hepatic porphryia. The chlorinated benzenes other than hexachlorobenzene had been shown in our previous studies to cause only minimal increases in liver or urinary porphyrins. Similarly, hexabromobenzene, 1,4-dibromobenzene, and 1,2,4-tribromobenzene (TBB) caused only small increases in hepatic porphyrins and did not cause increases in ALA synthetase, or the urinary excretion of porphobilinogen, aminolevulinic acid, or porphyrins.

Halogenated Aromatics

It was found that the trichlorophenols, which in some species are metabolites or contaminants in halogenated benzenes, were not inducers of xenobiotic metabolism. Addition of trichlorophenols *in vitro* showed they were inhibitors of both *p*-nitroanisole demethylation and EPN detoxification, in contrast to halogenated benzenes which had no *in vitro* effects. Also, the trichlorophenols were not hepatotoxic.

The brominated diphenyl ethers resemble PCBs and occur in commercial mixtures used as fire retardants. They are potent short-term inducers of xenobiotic metabolism, as well as potent long-term (90 days) inducers at low doses. Measurements of tissue content were not made, but it is likely that the extended period of induction, observable 30 to 60 days after the last dose, is a result of the potency of these agents as inducers and their accumulation in sites such as adipose tissue and liver.

Chlorinated diphenyl ethers were much inferior inducers of xenobiotic metabolism than predicted from studies on commercial brominated diphenyl ether mixtures. Diphenyl ether isomers with 2 or 3 chlorines were inactive at the experimental doses; however, similar polychlorinated biphenyls show low-inducing potential. Pentabromodiphenyl and octabromodiphenyl ether mixtures were more active than 4,4'-dibromodiphenyl ether. The more chlorinated the diphenyl ethers, the better their induction. Decachloro-diphenyl ether causes the greatest increases, except for AHH. This is in marked contrast to decabromodiphenyl ether which did not induce EPN

detoxification, p-nitroanisole demethylation, NADPH cytochrome c reductase, or cytochrome P-450. However, it resembles the observation of halogenated benzenes that hexachlorobenzene is a potent inducer and hexabromobenzene is not. Curiously, decachlorodiphenyl ether treatment caused a shift in the P-450 peak toward 448 but did not induce AHH, although 2,4,5,3',4'-pentachlorodiphenyl ether caused both effects. The degree of substitution and position of these groups are both important to determine the extent and type of induction.

The toxicity of the benzoquinones was found to be very cumulative. A dose that was far from being acutely toxic was lethal when given daily for 17 to 30 days. The reasons for this toxicity were not readily apparent, but one possibility considered and discarded as erroneous was that methemoglobin formation was occurring. There was no evidence of liver damage in this experiment, as measured by the sorbitol dehydrogenase in the blood. The cause of the cumulative toxicity remains a question. Both of the chlorinated dibenzofurans induced benzpyrene hydroxylase activity, but their parent compound dibenzofuran did not.

Relationship between Induction and Storage

Both TBB and TCB induce hepatic microsomal enzymes for at least 16 days after ending 7 days of a 1 mmol per kilogram dose per day. Excretion data indicated more TBB than TCB was held in the body. Metabolism of trihalogenated benzenes to trihalogenated phenols has been demonstrated in rabbits, but these are not inducers of xenobiotic metabolism in rats. The major metabolites in rats appear to be mercapturic acid derivatives.

Halogenated benzenes were observed in tissues. Increased induction from 4 days of starvation upon 6 days after TBB administration was terminated suggested TBB was mobilized from fat depots. Phenobarbital treatment immediately after halogenated benzene dosing seems to have hastened mobilization, metabolism, and excretion of either compound, leaving less in the rat to cause induction at the time of assay.

It is clear that both TBB and TCB are retained in the body and are slowly released after administration. Induction of hepatic microsomal enzymes after the 30-day recovery period following TBB dosing was not observed in our previous studies. This is most likely due to the considerably lower dosage used compared to TCB on a molar basis. On an equimolar basis, TBB leads to higher levels of hepatic enzyme induction that are maintained for longer periods and retained to a greater extent in body tissues. This compound is more influenced by starvation than is TCB. Starvation accelerates excretion of halogenated benzene. For phenobarbital treatment, this effect was inferred from the decrease in enzyme levels compared to those in non-phenobarbital-induced animals. A similar relationship of time and loss of elevated xenobiotic metabolism is seen for polychlorinated biphenyls. This induction is also increased by starvation.

Administration of the commercial polychlorinated biphenyl mixture, Aroclor 1254, resulted in increases in xenobiotic metabolism which persisted for as long as 4 to 5 months. Starvation caused an enhancement of this

induction, possibly due to a mobilization of material from body stores, as was observed for the halogenated benzenes.

Esterase Activity

Halogenated benzenes do induce xenobiotic metabolism by increasing esterase activity. This applies to phenyl acetate, acetanilide, and procaine. This indicates that they are broad inducers of oxidative, reductive, conjugative, and esteratic metabolic reactions. The compounds that were the most active in inducing arylesterase activity in the liver are 1,2,4-TCB and 1,2,4-TBB, shown throughout our studies as better inducing isomers.

Trihalogenated benzenes decreased the inhibitory effect of malathion on cholinesterase activity in the brain and to a lesser degree in red blood cells, but not in the liver or plasma. It, therefore, appears that halogenated benzenes protect against malathion or malaoxon toxicity by increasing the detoxification rate.

The trihalogenated benzenes decreased the inhibitory effect of paraoxon on cholinesterase activity in the brain and liver, but not in plasma or red blood cells. Thus, it appears that the halogenated benzenes protect against paraoxon and probably parathion toxicity by increasing the detoxification rate of the pesticides. The hepatic microsomal mixed function oxidase pathway for dearylation of paraoxon is a more important determinant of detoxification than the esterase pathway.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

SECTION 3

RECOMMENDATIONS

The results of these studies support our earlier findings that many of the halogenated aromatic compounds are capable of altering the metabolism of foreign organic compounds. These reactions include the mixed function oxidases, conjugative enzymes, and esterases. A critical area in need of additional research is the relationship of storage to prolonged induction.

In our studies on halogenated benzenes and polychlorinated biphenyls, the period of activity in increased xenobiotic metabolism lasted far beyond the period of administration of the compounds. The studies on halogenated benzenes were able to correlate this activity with storage, especially in adipose tissue. This is also suggested, but not proven, for PCBs. More studies on PCBs, as well as on brominated diphenyl ethers, are needed. Our findings that conditions such as starvation or administration of inducers of metabolism can alter the distribution and subsequently the effects of these lipophilic chemicals further substantiate the need for subsequent study.

The chlorinated benzofurans were found to be inducers, which supports previous findings of others. The chlorinated benzoquinones did not prove to be inducers. However, the toxicity of these agents proved to be highly cumulative. The reasons for this are not known, but are evidently not related to methemoglobin formation or liver damage. The dramatic nature of this cumulative toxicity, however, indicates that subsequent study is needed to ascertain the mechanism involved.

The brominated diphenyl ethers proved to be among the most potent inducers tested in our laboratory, however, little is known about their pharmacokinetic properties or effects other than those associated with xenobiotic metabolism. It is quite evident judging from their prolonged effects that they are stored in body tissues in ways similar to those of halogenated benzenes and polychlorinated biphenyls and then are slowly released. Additional studies are needed to quantify the tissue distribution, metabolism, and rate of elimination. These complex mixtures of isomers need to be identified to fully understand which are the active ones in causing the induction process. Finally, nothing appears to be known about the ability of these materials to cause porphyria. Studies would be prudent in view of their structural similarities to other porphyrinogenic compounds, such as polychlorinated biphenyls.

The lack of porphyria seen with the halogenated benzenes (other than the well-known porphyrinogenic agent, hexachlorobenzene) would suggest that

BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN, EPA FORM 287)
U.S. GOVERNMENT PRINTING OFFICE 1980-660-638
TYPING GUIDE SHEET

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
they do not as a class pose a threat to health. A conclusive recommendation to EPA is that further research is not warranted.
DROPPED HEAD; BEGIN SECTIONS HERE
6 1/8"
9 1/8"
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
8
PAGE NUMBER

EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)

☆ U.S. GOVERNMENT PRINTING OFFICE 1980-600-629

TYPING GUIDE SHEET

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

SECTION 4

MATERIALS AND METHODS

Sprague-Dawley rats (Laboratory Supply Co., Indianapolis, IN) were used for all experiments involving animals with the exception of Swiss mice (Laboratory Supply Co., Indianapolis, IN) being used to measure esterases and pesticide toxicity. Male rats (starting weight of 180-230 g) were used for most experiments. Female rats (starting weight of 120-140 g) are known to be more susceptible than males to porphyrinogenic effects of hexachlorobenzene and were used for the porphyrin studies. Pentabromodiphenyl ether and octabromodiphenyl ether used in one experiment were donated by Great Lakes Chemical Corporation, West Lafayette, IN. The laboratory in which the work was conducted was air-conditioned and light-controlled, and the animals were housed in stainless steel cages in the animal quarters, also temperature- and light-controlled.

Hepatic Porphyria

Groups of 5 female rats were administered 50, 100, or 200 mg/kg of 1,4-dibromobenzene, 1,2,4-tribromobenzene, or hexabromobenzene in corn oil daily po for 30, 60, 90, or 120 days. Controls received corn oil (1% v/w). Immediately after the last dose, the rats were placed in metabolism cages for 24-hour urine collection samples before being sacrificed. Livers were removed and portions prepared as 33 1/3% homogenates in 0.9% NaCl containing 0.5 mM EDTA and 10 mM Tris buffer pH 7.2. Samples were precipitated with 0.3 M trichloroacetic acid and centrifuged.

Urine samples or liver supernatants were passed through "piggy-back" ion exchange columns (Doss and Schmidt, 1971), and separations were carried out by washing with water and eluting the anionic column once with 2 ml of 1.0 N acetic acid and 2 ml of 0.2 N acetic acid (Piper et al., 1973) for porphobilinogen (PBG) measurement (Mauzerall and Granick, 1956). The column was then washed with 8 ml of 1.5 N HCl and porphyrins measured fluorometrically (excitation at 400 nm and emission at 600 nm) with coproporphyrin as the standard. The cationic column was eluted with 7 ml of 1.0 M sodium acetate and delta-aminolevulinic acid (ALA) determined (Doss and Schmidt, 1971). For the livers, only porphyrin content was measured. Portions of the liver homogenate were also used to measure ALA synthetase activity (Goldstein et al., 1974). Comparisons among the dose levels were made using Duncan's new multiple range test.

BEGIN LAST LINE OF TEXT
3"/8"

PAGE NUMBER
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
© U.S. GOVERNMENT PRINTING OFFICE 1980-680-639
TYPING GUIDE SHEET

Halogenated Aromatics

Chlorinated Phenols--

The method used to evaluate trichlorophenols involved groups of 6 animals that were administered 25, 100, or 400 mg/kg of 2,3,5-, 2,4,5-, or 2,4,6-trichlorophenol or 25, 100, or 200 mg/kg of 2,3,6-trichlorophenol by intubation daily for 14 days. Controls received corn oil which was used as the vehicle for the trichlorophenols. The animals were lightly anesthetized with ether 24 hours after the last dose. A small portion of the liver was removed to determine EPN detoxification (Kinoshita et al., 1966). The livers were perfused in situ with cold isotonic saline. Microsomal fractions were prepared. NADPH-cytochrome c reductase activity and cytochrome P-450 content were measured (Dallner, 1963), as were proteins (Lowry et al., 1951).

For in vitro studies, chlorinated phenols were dissolved in benzene and added so 50 µl gave a final concentration of 2.5×10^{-4} M. Benzene had no significant effect on activity. A determination of p-nitroanisole demethylation was made. To decide if the inhibition was competitive or noncompetitive, p-nitroanisole demethylation assays (Kinoshita et al., 1966) were conducted for 15 minutes with substrate concentrations of 0.4, 0.6, 1.0, and 2.0 mM, and 2,3,5-trichlorophenol at a final concentration of 0.1 mM.

The influence of the compounds on glucuronyltransferase activity was assessed following both in vivo and in vitro administration of the compounds. Groups of 5 rats received 200 mg/kg daily for 14 days po. Controls received corn oil. Microsomes were prepared and glucuronyltransferase activity measured using naphthol as the substrate (Lucier et al., 1971). In vitro the compounds were dissolved in benzene and added to give a final concentration of 1×10^{-4} M. The control sample received the equivalent volume of benzene.

Initial studies indicated the compounds might be toxic to the liver. To assess hepatotoxicity, each of the 4 trichlorophenols was administered at a dose of 200 mg/kg po to groups of 4 rats daily for 14 days. Lightly anesthetized with ether, the animals had tail vein blood samples drawn for measurement of serum sorbitol dehydrogenase (Gerlach, 1965). The rats were sacrificed and portions of the livers removed for glucose-6-phosphatase determination (Harper, 1965).

In the in vivo studies, comparisons were made among doses using Duncan's new multiple range finding test (Steel and Torrie, 1960). For in vitro, a paired Student's t-test was used. For both, the level of significance chosen was $P<0.05$.

Brominated Diphenyl Ethers--

Brominated diphenyl ethers were investigated by administering to groups of male rats weighing 200 to 250 g 0.1 mmol/kg/day po of the materials in corn oil for 14 days. Diphenyl ether (98%) and bis(p-bromodiphenyl)ether (reagent grade) were purchased from Eastman Chemical Company (Rochester, NY).

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
U.S. GOVERNMENT PRINTING OFFICE: 1980-660-039
TYPING GUIDE SHEET

Pentabromodiphenyl ether and decabromodiphenyl ether were also used. Twenty-four hours after the seventh dose, the animals were sacrificed and their livers removed and weighed. The EPN detoxification and p-nitroanisole demethylation were determined. When NADPH cytochrome c reductase and cytochrome P-450 were measured, the livers were perfused in situ with cold isotonic saline and microsomal fractions prepared. UDP-glucuronyltransferase activity using naphthol as the substrate and benzpyrene hydroxylase (Nebert and Gelboin, 1968) were also determined in microsomal fractions, as were proteins. Tail vein blood samples were obtained and used for serum sorbitol dehydrogenase measurements.

Values are expressed as mean ± S.E. Duncan's new multiple range finding test was used to compare all treatments among themselves and with control. The level of significance chosen was $P<0.05$.

Pentabromodiphenyl ether and octabromodiphenyl ether are commercial mixtures, with their degree of bromination identified. These compounds were administered po to rats weighing 200 to 250 grams daily for 90 days. When original doses of 6.25, 12.5, and 25 µmol/kg/day revealed extensive induction even at the lowest dose, the experiment was repeated at doses of 0.78, 1.56, and 3.13 µmol/kg daily.

Animals were lightly anesthetized with ether 1, 30, or 60 days after the last dose and portions of their livers removed for fixation in buffered formalin and staining with hematoxylin and eosin. They were histologically evaluated. Another portion was used for measurement of EPN detoxification and p-nitroanisole demethylation. The remaining liver was perfused in situ with cold isotonic saline and microsomal fractions were prepared. Measurements of NADPH cytochrome c reductase and cytochrome P-450, and also proteins, were made.

Chlorinated Diphenyl Ethers--

Chlorinated diphenyl ethers were dissolved in corn oil and administered to groups of 5 adult male rats at a dose of 10 µmols/kg daily for 3 days. The parent molecule, diphenyl ether, was included at the same dosage level as the chlorinated isomers. Controls were given corn oil. The animals were sacrificed 24 hours after the last dose.

Lightly anesthetized with ether, portions of the animals' livers were removed for measurement of EPN detoxification. A second portion was removed for measurement of microsomal aryl hydrocarbon hydroxylase (AHH). The livers were perfused in situ with cold isotonic saline and microsomal fractions prepared by centrifuging at 9,000 x g for 20 minutes, followed by centrifugation of the supernatant at 105,000 x g for 60 minutes in a Beckman L5-65 ultracentrifuge. Using a Beckman ACTA-CIII spectrophotometer, NADPH cytochrome c reductase activity and cytochrome P-450 content were determined. The wavelength for maximum absorption of the cytochrome P-450 was also determined and the proteins were measured.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN: EPA FORM 2327)
© U.S. GOVERNMENT PRINTING OFFICE 1980-650-029
TYPING GUIDE SHEET

Benzoquinones--

To determine activity of benzoquinones, 2,5-dichloro-p-benzoquinone was administered at doses of 20 and 100 mg/kg po or ip to examine cumulative toxicity. Groups of 5 rats received doses daily for up to 30 days. Benzoquinone toxicity could be due to either methemoglobin formation or to liver damage, so rats were administered p-benzoquinone, 2,5-dichloro-p-benzoquinone, 2,6-dichloro-p-benzoquinone, or 2,5-dimethyl-p-benzoquinone at a dose of 12.5 mg/kg daily for 14 days po. Methemoglobin (Bauer, 1970) and sorbital dehydrogenase were measured in blood and serum from animals sacrificed 24 hours after their last dose.

Graded doses of substituted benzoquinones were administered for 14 days to groups of 6 to 8 rats to study the effects of benzoquinones on xenobiotic metabolism. Although many animals died, EPN detoxification, NADPH cytochrome c reductase, and cytochrome P-450 content were measured using the customary procedures in those animals surviving the 14 days of treatment.

Dibenzofurans--

The dibenzofurans were studied with groups of 5 rats, each administered 5 µmols/kg daily for 3 days of dibenzofuran, 2,8-dichlorodibenzofuran, or octachlorodibenzofuran. The animals were sacrificed 24 hours after the last dose and EPN detoxification, benzpyrene hydroxylase, NADPH cytochrome c reductase, and cytochrome P-450 were measured using the customary procedures.

Relationship between Induction and Storage

This relationship was studied by a combination of radionuclide compound usage to assess distribution and starvation periods to measure changes due to alteration of storage in groups of male rats. In the first set of experiments, animals received reagent grade TBB or TCB at 1 mmol/kg daily po for 7 days in corn oil as 0.1% of body weight. Control animals received an equivalent volume of corn oil. The animals were sacrificed by decapitation and a portion of liver was removed for measurement of the detoxification of EPN to p-nitrophenol, one of the most sensitive indicators of induction by these compounds and O-demethylation of p-nitroanisole. The remaining liver was perfused via the portal vein with cold 0.90% NaCl and microsomal fractions were prepared.

Urinary and fecal excretion of ^{14}C-labeled TBB or TCB and metabolites was measured by liquid scintillation counting of samples of urine and feces collected from rats in individual metabolism cages. For these studies, 2 µCi/kg daily was administered. Of the total 24-hour sample of dried, powdered feces, 2 grams were extracted with 8 ml benzene. A sample (0.5 ml) was then counted. Radioactivity was determined in a liquid scintillation counter. The scintillation medium was 6 grams of 2,5-diphenyloxazole (PPO) and 75 mg 1,4-bis(2,5-phenyloxazolyl)benzene (POPOP) dissolved in 1 liter toluene. Quenching was determined by using an internal standard.

Tissue samples were homogenized in 4 volumes of water, and 0.3 ml homogenate was added to a vial containing 1.8 ml NCS tissue solubilizer for

analysis of tissue ^{14}C levels. Samples were digested at 50°C until clear and were then cooled. The scintillator was added.

To study the effect of starvation, groups of rats received 1 mmol/kg daily of TCB or TBB for 7 days. Controls received corn oil. All animals had a recovery period of 6 days. Food (but not water) was removed from half of each group for the next 4 days. When phenobarbital was used, the animals were dosed with TCB or TBB, as usual. After the seventh dose, half of the treated animals and half of the controls were injected daily for 4 days with sodium phenobarbital (80 mg/kg, ip). All animals had a recovery period of 6 days before being sacrificed for various measurements.

Duration of Induction by PCB and Influence of Starvation--

Groups of 5 rats were administered 10 mg/kg daily of Aroclor 1254 po for 14 days. Controls received corn oil. At various time periods, groups were sacrificed and portions of liver removed for measurement of EPN detoxification and p-nitroanisole demethylation. The remaining liver was perfused with cold saline and microsomal fractions prepared. Microsomal NADPH cytochrome c reductase activity and cytochrome P-450 (including P-448) content were measured and protein concentrations determined. In some cases, animals were starved for 4 days prior to sacrifice.

Esterases

Arylesterase--

The arylesterase activity was measured using phenyl acetate as the substrate (Lorentz et al., 1979). Groups of 5 rats were treated with 0.1 mmol/kg daily of 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, hexachlorobenzene, 1,2,4-tribromobenzene, 1,3,5-tribromobenzene, or hexabromobenzene po for 14 days. Blood samples were taken for serum preparation by centrifugation, and a portion of the liver was homogenized and centrifuged at 9,000 x g. The amount of tissue was 0.2 µl of serum or 0.2 mg equivalent of the whole liver as the 9,000 x g fraction.

Acetanilide and Procaine Esterases--

To analyze effects of halogenated benzenes on acetanilide hydroxylase and esterase, 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, hexachlorobenzene, and corresponding bromine analogs were administered in corn oil po daily at a dose of 0.1 mmol/kg daily for 14 days. The acetanilide hydroxylase reaction mixture (Krisch and Staudinger, 1961) contained 4 µmols of Tris·HCl buffer (pH 8.2), 10 µmols of $MgCl_2 \cdot 6H_2O$, 2 µmols of NADPH and microsomes equivalent to 200 mg liver in a total volume of 1.0 ml. The reaction was carried out at 37°C for 15 minutes and was stopped by 0.1 ml of 20% trichloroacetic acid. The contents were analyzed for p-hydroxyacetanilide. Acetanilide esterase activity was measured (Akao and Omura, 1972) with Tris·HCl buffer (pH 8.6), microsomes equivalent to 10 mg liver, and a 15 minute incubation period.

Procaine esterase activity was assessed after administration of 1 mmol/kg daily po for 14 days. The assay of the final incubation mixture (Cohen et al., 1977) consisted of 2 ml of 0.067 M sodium phosphate buffer (pH 7.2) and 2 ml of whole homogenate (10% w/v in buffer). The incubation time was 2 hours. Proteins were determined.

Esterases and Pesticide Toxicity--

To measure esterases and their relationship to parathion, malathion, paraoxon, and malaoxon toxicity, male, Swiss mice (Laboratory Supply Co., Indianapolis, IN) weighing 25 to 30 grams were used. They were administered 1 mmol/kg daily po of 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, 1,4-dibromobenzene, 1,2,4-tribromobenzene, hexabromobenzene, or 0.1 mmol/kg of hexachlorobenzene daily for 7 days. LD50s were determined by treating the animals for 7 days and administering graded log doses of malathion, malaoxon, parathion, or paraoxon po. Within 24 hours after administration of the pesticide, the number of dead animals was determined and the LD50s calculated (Finney, 1971).

Cholinesterase activity in the brain, liver, plasma, and red blood cells was determined by measuring the hydrolysis rate of acetylthiocholine spectrophotometrically (Ellman, 1961). Mice were administered 1 mmol/kg daily of trichlorobenzene or tribromobenzene po for 7 days. On day 8, animals received either 1 g/kg malathion or 10 mg/kg paraoxon po in corn oil. Controls received only corn oil. Blood samples were drawn by orbital sinus puncture 1 to 2 hours later, and portions of the liver and the whole brain were obtained for cholinesterase activity determination.

Carboxylesterase activity was measured in liver and plasma of mice spectrophotometrically (Talcott, 1979). The mice were treated for 7 days, as described above. Blood was drawn from the orbital sinus and a portion of liver removed for determination.

Paraoxon dearylation to p-nitrophenol was measured spectrophotometrically (Villeneuve, 1970; Cammer and Hollingworth, 1976). Mice were pretreated with halogenated benzenes in the customary manner. On day 8, blood and liver samples were obtained. Plasma was prepared by centrifugation. The liver was centrifuged at 9,000 x g for 20 minutes and the supernatant at 105,000 x g to prepare soluble and microsomal fractions.

Esterases in the plasma and soluble fractions and microsomes were measured by incubating the tissue with 0.5 mM paraoxon in Tris buffer (pH 7.6). The microsomal mixed function oxidase dearylation activity was measured by incubating with 0.1 mM EDTA to inhibit esterase activity and 1.2 mM NADPH as the cofactor. The measurement of activity was by the amount of p-nitrophenol formed.

SECTION 5

RESULTS

Basically xenobiotic metabolism is induced by chlorinated and brominated benzenes. The induction affects both mixed function oxidases and esterases. The second major finding is that porphyria is not caused by chlorinated benzenes, except for hexachlorobenzene. Since the study has been broader and more detailed than originally proposed, there are a variety of major and minor results from experiments.

Hepatic Porphyria

Hexachlorobenzene does greatly increase the production and excretion of porphyrins (Carlson, 1977), but hexabromobenzene has very little effect (Table 1). There was only a 42% increase in porphyrin content of the liver and no increased excretion of porphyrins in the urine even at a dose of 200 mg/kg daily for 120 days. No increases were observed in ALA synthetase or the excretion of ALA or PBG in the urine. 1,4-Dibromobenzene also was not very porphyrinogenic (Table 2). The highest increase in liver porphyrin content was approximately double. It was slightly more potent than the chlorine derivative. If enlargement of the liver is considered, the increase is amplified. There was no increase in urinary excretion of porphyrins, PBG, or ALA, or in the hepatic synthesis of ALA.

Administration of 1,2,4-tribromobenzene caused a significant increase in liver weight, even at the lowest level of 50 mg/kg in 30 days. An increase in porphyrin content in the liver did not appear until 90 days (Table 3). The increases were dose dependent but were small and very similar to those observed with 1,2,4-trichlorobenzene. As with other compounds in this project, increased production of porphyrins was not accompanied by an increase in excretion, and there were no increases in hepatic ALA synthetase activity or the urinary excretion of PBG or ALA.

Animals treated with 200 mg/kg of 1,2,4-tribromobenzene for 120 days appeared to be slightly smaller than the controls, but the difference was not significant. They were discolored and their ears were especially brown and ragged in appearance. Thus, although the animals did not appear healthy, porphyria did not appear to be much of a problem.

The results indicated that hexabromobenzene does not share the extreme porphyrinogenic properties of its chlorinated analog. The less brominated compounds are similar to the chlorinated analogs in being very weak in ability to induce porphyria. Thus, the data would indicate that, unlike hexachlorobenzene, porphyria would not be a significant hazard associated with intake of these compounds.

Halogenated Aromatics

Chlorinated Phenols--

All of the experimentation resulted in much negative data for halogenated aromatic induction of xenobiotic metabolism. They did not induce EPN detoxification, NADPH cytochrome c reductase, or cytochrome P-450 (Table 4). It is clear that compounds such as 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene do not depend upon biotransformation to trichlorophenols for inducing ability, so they must be regarded as inactive metabolites.

In vitro the trichlorophenols were inhibitors of EPN detoxification and p-nitroanisole demethylation (Table 5). At least for p-nitroanisole demethylation, the inhibition was noncompetitive. The 2,3,5- and 2,4-5-isomers were the most potent.

These compounds did not share the ability of pentachlorophenol (Arrhenius et al., 1977) to induce glucuronyltransferase when administered in vivo (Table 5) and in vitro have an inhibitory nature (Table 7). These compounds, it must be noted, were not hepatotoxic (Table 8).

Brominated Diphenyl Ethers--

It was found that all of the brominated diphenyl ethers, including the commercial pentabromodiphenyl and octabromodiphenyl ethers, which are complex mixtures (Table 9), caused liver enlargement when liver to body weight ratios were determined (Table 10). Pentabromodiphenyl ether gave the largest increase of 64%. A similar pattern was seen with both NADPH cytochrome c reductase and cytochrome P-450 with pentabromodiphenyl ether. The effect of pentabromodiphenyl ether is greater than that of octabromodiphenyl ether, which is equal to the effect of bis(p-bromodiphenyl) ether. Decabromodiphenyl ether was without effect. This is similar to the poor inducing properties of fully brominated benzene.

When a series of enzyme activities was determined, pentabromodiphenyl ether and octabromodiphenyl ether were the greater inducers, giving large increases in both EPN detoxification and p-nitroanisole demethylation (Table 11). They were the only treatments which gave increases in conjugation of naphthol. Benzo(a)pyrene hydroxylase was increased only by pentabromodiphenyl ether. Diphenyl ether caused an increase in EPN detoxification, which is similar to the weak inducing properties of biphenyl compared to polychlorinated biphenyls (Johnstone et al., 1974).

The activity of bis(p-bromodiphenyl) ether appeared to the intermediate between the nonbrominated and more brominated diphenyl ethers. However, decabromodiphenyl ether did not increase any of the four systems. Sorbitol dehydrogenase activity in the serum was not elevated in any of the groups, which indicated a lack of liver damage by these compounds at the doses used.

Administration of 6.25, 12.5, or 25 μmol/kg daily of the pentabromodiphenyl ether and octabromodiphenyl ether over a 90-day period resulted in

large increases in EPN detoxification, p-nitroanisole demethylation, and cytochrome P-450 (Table 12). Pentabromodiphenyl ether did not cause large increases in NADPH cytochrome c reductase, and octabromodiphenyl ether did not cause significant increases, even at the highest dose level.

When administered for 90 days, even the lowest dose tested of pentabromodiphenyl ether (0.78 µmol/kg daily) caused increases in EPN detoxification, p-nitroanisole demethylation, NADPH cytochrome c reductase, and cytochrome P-450 (Table 13). For EPN detoxification and p-nitroanisole demethylation, there was a clear-cut dose response relationship. When the animals were given a 30-day recovery period following the 90th dose, elevations in these two measurements were still observable at all doses, although only with the highest dose did NADPH cytochrome c reductase activity increase. Within this time period, cytochrome P-450 content had returned to control level. Sixty days after the last dose, slight elevations were noted in EPN detoxification and p-nitroanisole demethylation at the two higher levels (1.56 µmol/kg daily and 3.13 µmol/kg daily).

Octabromodiphenyl ether caused no induction of NADPH cytochrome c reductase and cytochrome P-450 at these lower doses (Table 14). A dose of 0.78 µmol/kg daily caused increases in both EPN detoxification and p-nitroanisole demethylation, and still larger increases were seen with increasing doses. After a 30-day recovery period, there was evidence of the maintenance of an induced state only in the animals receiving the highest dose of 3.13 µmol/kg daily. These elevations were still observable 60 days after the last dose.

No abnormalities were observed when the livers from the rats treated with brominated diphenyl ethers in the low-dose study were examined by light microscopy.

Chlorinated Diphenyl Ethers--

Chlorinated isomers proved poor inducers of xenobiotic metabolism when compared with the very potent inducing ability of the polybrominated diphenyl ethers. Only 2,4,5,2',4'-pentachlorodiphenyl ether and the fully substituted decachlorodiphenyl ether caused increases in EPN detoxification, and the latter increase was double (Figure 1). There was more variation with determination of aryl hydrocarbon hydroxylase activity than with EPN detoxification. It is clear that only two of the isomers, 3,4,2',4'-tetrachlorodiphenyl ether and 2,4,5,3',4'-pentachlorodiphenyl ether, caused increases (Figure 2). Decachlorodiphenyl ether was ineffective in inducing AHH activity despite its high activity in increasing EPN detoxification.

When microsomal NADPH cytochrome c reductase activity was measured, only decachlorodiphenyl ether caused an increase (Figure 3). This compound caused the largest increase in cytochrome P-450 content, although small but significant elevations were also observed with rats treated with both 2,4,5,2',4'- and 2,4,5,3',4'-pentachlorodiphenyl ether (Figure 4). It was found that only two of the isomers, 2,4,5,3',4'-pentachlorodiphenyl ether and decachlorodiphenyl ether, caused a shift from 450 nm to 448.2 nm and 448.4 nm, respectively, when the peak of maximum absorbance was determined.

Benzoquinones--

The toxicity of the benzoquinones proved very cumulative. Although a dose of 20 mg/kg daily of 2,4-dichloro-p-benzoquinone po was far from being acutely toxic, administration of this dose daily for 17 or 30 days proved lethal (Table 15). At 100 mg/kg daily, animals were able to survive administration of the compound for four days before they began dying. Oral toxicity was less than that seen with intraperitoneal administration.

The reason for benzoquinone toxicity was not readily apparent. One possibility is that methemoglobin formation was occurring, but it was found that p-benzoquinone, 2,5-dichloro-p-benzoquinone, 2,6-dichloro-p-benzoquinone, and 2,5-dimethyl-p-benzoquinone did not cause methemoglobinemia (Table 16). In the same study, there was no evidence of liver damage, as assessed by measurement of serum sorbitol dehydrogenase. Although many halogenated aromatic compounds studied have induced xenobiotic metabolism, the benzoquinones did not alter EPN detoxification, NADPH cytochrome c reductase, or cytochrome P-450 (Table 17).

Dibenzofurans--

Dibenzofuran or its chlorinated derivatives did not increase EPN detoxification and NADPH cytochrome c reductase (Table 18). However, benzpyrene hydroxylase activity was induced by both chlorinated dibenzofurans but not by the parent compound. Both chlorinated dibenzofurans appeared to increase cytochrome P-450 content, although the increase was not statistically significant in the case of the 2,8-dichloro isomer. Both also caused a shift to 448.2 nm for the peak of cytochrome P-450 absorbance. Those factors strongly support the contention that these compounds are inducers of the 3-methylcholanthrene type.

Relationship between Storage and Induction

Halogenated Benzenes--

There is a large difference in molecular weight in the halogenated benzenes tested previously (Carlson and Tardiff, 1976, 1977), and for this reason, they were compared on a molar basis. A dose of 1 mmol/kg daily for 7 days was used in all studies. This dose caused a high level of induction on day 1 after the last dose of either TCB or TBB (Figures 5 and 6). EPN detoxification levels were approximately twice as high as control values 16 days after the last dose of TBB suggesting induction may have occurred beyond day 16 of recovery. The decline in induction appeared slower with TBB than with TCB.

Results with the p-nitroanisole demethylation assay closely paralleled those of EPN detoxification for both TBB and TCB treatments. NADPH-cytochrome c reductase activity and cytochrome P-450 content declined more rapidly after cessation of dosing. Significant induction was observed only through days 6 and 11 after termination of dose, respectively, for TBB and TCB.

It was important to establish that the compounds were being stored because induction was demonstrated 16 days after dosage termination. This was studied with ^{14}C-labeled TBB and TCB (2 µCi/kg daily). The highest concentration of either halogenated benzene was found in the fat (Table 19). The adrenals initially had greater ^{14}C concentrations than fat, but the levels declined rapidly. Tissue levels of ^{14}C in the TBB-treated group were often at least twice those in the TCB-treated animals. Levels of both compounds declined by day 6 and further by day 11.

Excretion of ^{14}C-labeled material was investigated during the 7 days of dosing, for 8 days after the last dose for fecal excretion, and for 15 days after the last dose for urinary excretion (Figures 7 and 8). Fecal excretion after TBB or TCB dosing amounted to approximately 10 and 4%, respectively, of total excretion. For both compounds, fecal excretion decreased very rapidly after cessation of administration. Activity of ^{14}C was not measurable in fecal samples beyond day 8 of recovery. In contrast, urinary excretion was still measurable 15 days after termination of dosing. About 1.4 times as much ^{14}C was excreted from animals receiving TCB than from animals dosed with TBB. In other terms, almost twice as much ^{14}C remained in the body in TBB-treated animals compared to TCB-treated ones.

It was determined whether or not starvation might mobilize stored TCB or TBB and alter the decline in enzyme levels. Four days of starvation after 7 days of dosing with TBB and 6 days of recovery resulted in significantly greater increases in induction of hepatic microsomal enzymes than were observed in fed TBB-treated rats (Table 20). Starvation alone did not cause changes in enzymes that were observable at the time of assay. No increase in induction due to starvation was observed for rats receiving TCB (Table 21).

These observations indicate that TBB but not TCB was mobilized from tissues to cause this induction. Therefore, starvation would be expected to cause increased plasma levels of TBB but not TCB. Four days of starvation after 7 days of TBB dosing and 6 days of recovery significantly increased the concentration of ^{14}C in the plasma of rats. Plasma levels of ^{14}C were too low to be adequately determined in either the starved or fed TCB-treated groups. Samples of abdominal fat and liver tissue were also analyzed. The TBB-treated, starved animals had significantly higher concentrations of ^{14}C in both fat and liver tissue than the TBB-treated, fed animals (Table 22). No significant change was observed in ^{14}C levels of the TCB-treated rats due to the 4 days of starvation.

Increased ^{14}C in the blood of TBB-treated animals would be expected to cause increases in urinary excretion of this compound. Because of the low levels of ^{14}C present in the urine from the TCB-treated rats, only the TBB data were analyzed statistically. No differences in ^{14}C excretion were observed for the last 2 days of recovery after TBB dosing, indicating that the two groups were similar before starvation (Table 23). Significantly higher levels of ^{14}C were excreted in the urine during the first 2 days of starvation, and increases were seen on days 3 and 4, but these were not statistically significant.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
3-8"
BOTTOM OF IMAGE AREA. OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (1-80)
(PREVIOUSLY CIN, EPA FORM 2911)
TYPING GUIDE SHEET

Phenobarbital has induced hepatic microsomal metabolism and thereby hastened removal of xenobiotics from the body (Lambert and Brodeur, 1976). Whether phenobarbital, administered when fat stores of halogenated benzene were high, would decrease induction caused by TBB or TCB was studied. Phenobarbital induction was not expected to be maintained throughout the 6-day recovery period. Generally, induction with TBB or TCB was observable at time of assay, and the phenobarbital effect was usually insignificant (Figures 9 and 10). A two-way ANOVA indicated a significant interaction between TBB and phenobarbital treatments for p-nitroanisole demethylation activity and cytochrome P-450 content. The value for the combination was lower than the value for either treatment alone. An interaction was also noted between TCB and phenobarbital for all three assays.

Duration of Induction by PCB and Influence of Starvation--

The results with administration of Aroclor 1254 were as expected. EPN detoxification and p-nitroanisole demethylation were elevated four- and tenfold, respectively, 1 day after the last (14th) dose of Aroclor 1254 (Table 24). Two- to fourfold increases due to Aroclor alone were generally seen up to approximately 71 days. After that the increases declined so that by 120 and 148 days after the last dose, EPN detoxification was only slightly elevated and p-nitroanisole demethylation had returned to control level. When control rats were starved for 4 days, it did not affect either of the two parameters. However, when Aroclor-treated rats were starved for 4 days, there was an increase in these enzyme activities over the activity observed with Aroclor alone and no starvation. These increases were still quite marked at 106 days, but were minimal and observed only with the p-nitroanisole demethylation at 120 and 148 days.

When measurements were done with microsomal NADPH cytochrome c reductase and cytochrome P-450, similar results were found (Table 25). Cytochrome P-450 increases were observed for the first 71 days due to Aroclor alone before returning to control levels at later time periods. Changes in NADPH cytochrome c reductase, although not as large, followed a similar time course. Starvation had no effect on these measurements in the control groups. Starvation of the Aroclor-treated animals resulted in enhancement of the inducing effects of the Aroclor and, as before, this interaction disappeared by 120 days.

Esterase Activity

Acetanilide and Procaine Esterases--

Halogenated benzenes induce the metabolism of acetanilide and procaine by increasing esterase activity (Tables 26 and 27). This is true of the less halogenated benzenes as well as for hexachlorobenzene. It is found that these compounds are broad inducers of oxidative, reductive, conjugative, and esteratic metabolic reactions.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

Esterases and Pesticide Toxicity--

The 1,4-dihalogenated benzenes and hexabromobenzene had little effect on the lethality of malathion (Table 28). Both trihalogenated benzenes increased the LD50 values, which indicated a decrease in lethality. The trihalogenated benzenes were also able to decrease toxicity of the active metabolite of malathion, malaoxon, approximately twofold (Table 29). Even greater protection against lethality was demonstrated with parathion (Table 30). Although 1,4-dichlorobenzene and hexachlorobenzene were without effect, 1,4-dibromobenzene, which had little effect on malathion toxicity, increased the LD50 of parathion twofold. The LD50 was increased fourfold and sixfold, respectively, over the control by 1,2,4-trichlorobenzene and 1,2,4-tribromobenzene. Paraoxon findings were similar (Table 31). There was little effect with 1,4-dichlorobenzene and hexachlorobenzene, but 1,4-dibromobenzene increased the LD50 twofold and both halogenated benzenes increased it still more.

The effect of the trihalogenated benzenes on cholinesterase activity in the brain, liver, plasma, and red blood cells was measured, since toxicity of the organophosphates is related to inhibition of cholinesterase. Malathion caused a decrease in cholinesterase activity in the brains of control mice but not in those treated with 1,2,4-trichlorobenzene (Table 32). The decreases were similar between control and treated groups in other tissues. Similarly, 1,2,4-tribromobenzene treatment prevented the decrease in brain cholinesterase following malathion treatment and slightly decreased the malathion effect on red blood cells. It was without effect on the liver or plasma (Table 33).

Measurement of carboxylesterase activity with malathion as the substrate revealed that 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, hexachlorobenzene, 1,4-dibromobenzene, 1,2,4-tribromobenzene, and hexabromobenzene all increased carboxylesterase activity in the liver (Table 34). A similar trend was seen in plasma. The trihalogenated benzenes caused the largest increases in activity. In carboxylesterase studies with malaoxon as the substrate, no elevations of activity in the liver were seen with any of the treatments (Table 35). In plasma, only the trihalogenated benzenes were able to slightly increase the activity of the enzyme.

Paraoxon was administered and effects on cholinesterase activity were determined, similar to the malathion experiments. Treatment with 1,2,4-trichlorobenzene prevented the decrease in brain cholinesterase due to this organophosphate (Table 36). There was partial protection in the liver, but decreases were similar in the control and treated animals for plasma and red blood cells. Similar observations were made with 1,2,4-tribromobenzene treatment (Table 37). Protection was found in the brain, to some extent in the liver, but none was noted in serum or red blood cells.

Paraoxon dearylation was examined since it was suspected that halogenated benzenes might be protecting against paraoxon toxicity by increasing its metabolism. Plasma esterase was not altered by treatment with 1,4-dihalogenated or hexahalogenated isomers, and trihalogenated isomers actually decreased activity (Table 38). As expected, there was little activity in

BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 2817)
☆ U.S. GOVERNMENT PRINTING OFFICE: 1980-660-639
TYPING GUIDE SHEET

either control or treated animals in the 105,000 x g liver supernatant. Microsomal esterase activity was increased by 1,2,4-trichlorobenzene, hexachlorobenzene, and hexabromobenzene, but it was inhibited by 1,4-dibromobenzene and 1,2,4-tribromobenzene.

The mixed function oxidase dearylation in the microsomes was increased slightly by 1,4-dichlorobenzene, hexachlorobenzene, and 1,2,4-chlorobenzene. Treatment with 1,2,4-tribromobenzene resulted in an eightfold increase. Therefore, the best correlation between protection against lethality by the various isomers and increases in detoxification of paraoxon is related to changes in mixed function oxidase activity of the liver and not to changes in the esterases in the plasma or liver.

Arylesterase--

When arylesterase activity was measured in the serum, none of the halogenated benzenes tested caused any increase in activity (Table 39). They included 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, hexachlorobenzene, 1,2,4-tribromobenzene, 1,3,5-tribromobenzene, and hexabromobenzene. Increases in activity were observed in the liver after administration of 1,2,4-trichlorobenzene, hexachlorobenzene, 1,2,4-tribromobenzene, and 1,3,5-tribromobenzene. The compounds, therefore, associated throughout this research project as the better inducing isomers were also the most active in inducing arylesterase activity in the liver.

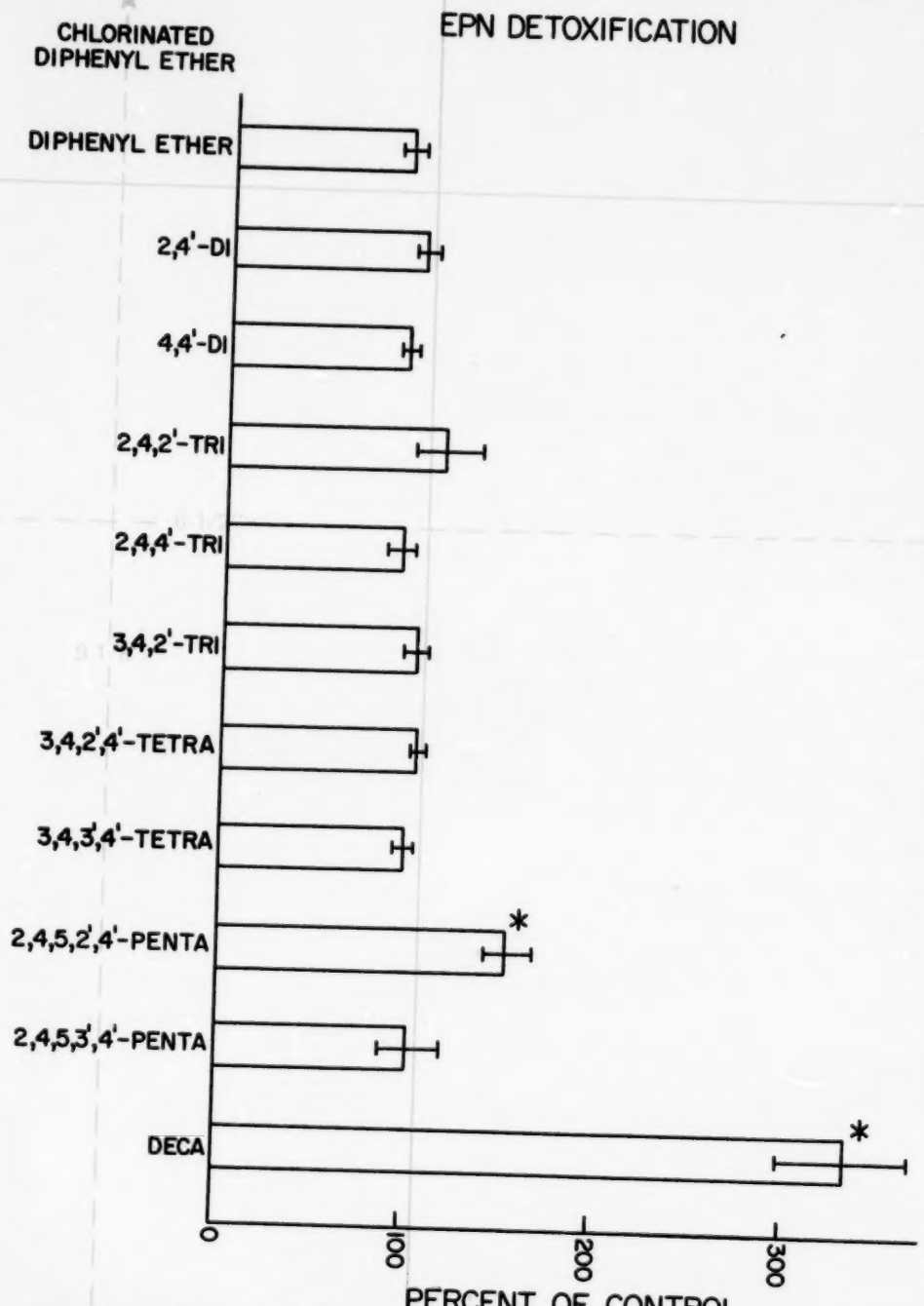

FIGURE 1. Effect of 10 µmols/kg/day of chlorinated diphenyl ethers for 3 days po on EPN detoxification. Asterisk indicates a result significantly different from control ($P<0.05$). Average control value is 6.51 µg p-nitrophenol formed/30 min/50 mg.

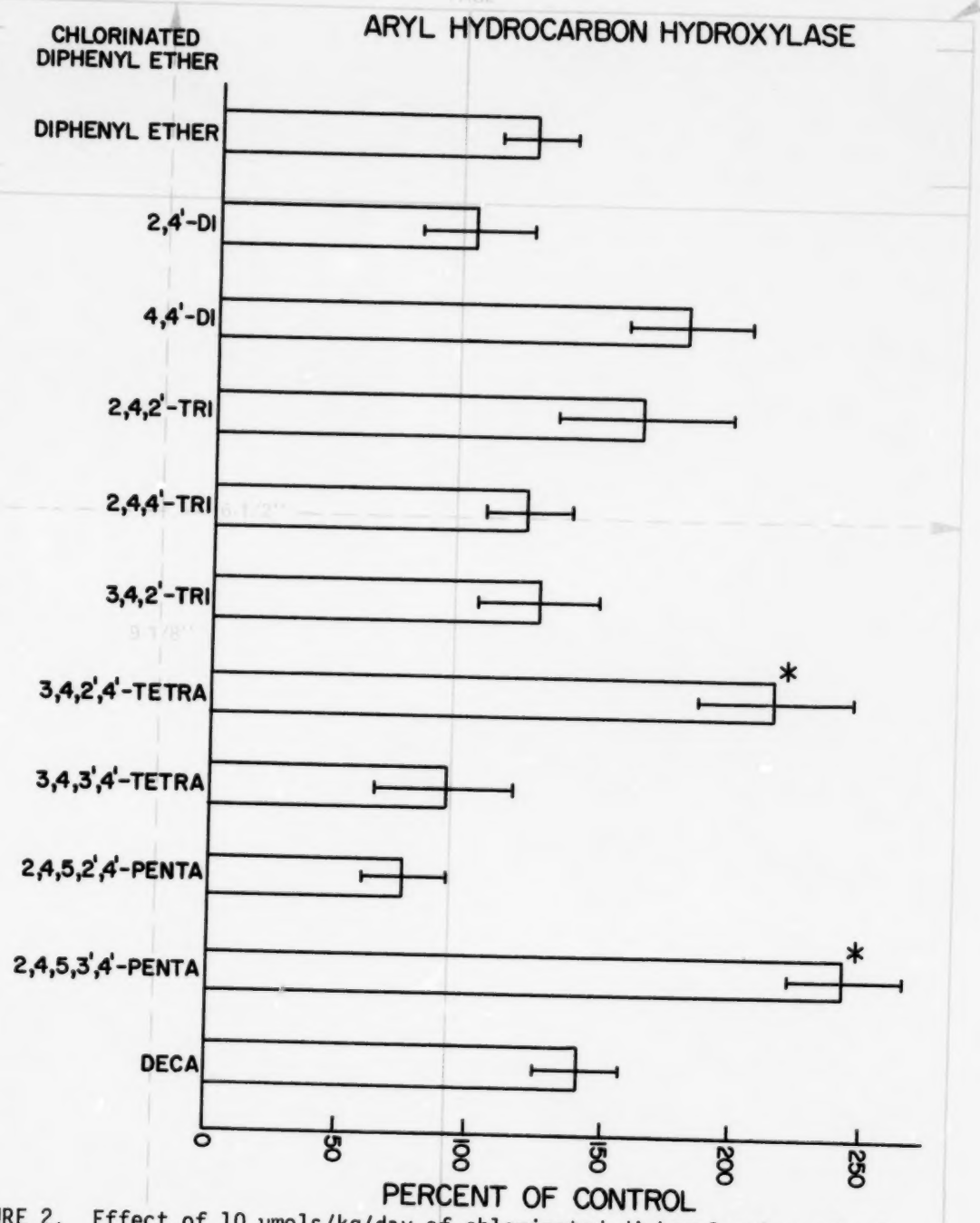

FIGURE 2. Effect of 10 µmols/kg/day of chlorinated diphenyl ethers for 3 days po on aryl hydrocarbon hydroxylase. Asterisk indicates a result significantly different from control ($P<0.05$). Average control value is 4.92 nmols/10 min/mg protein.

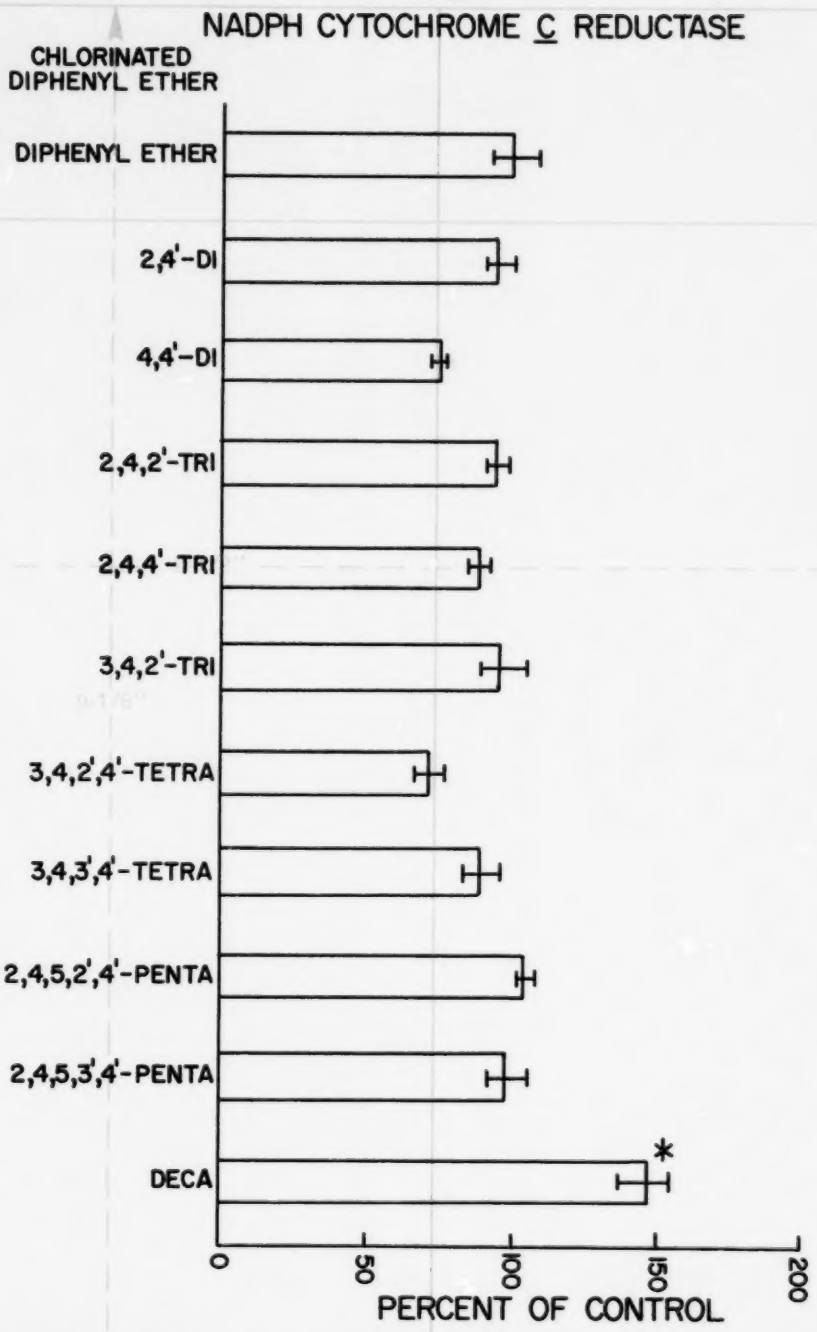

FIGURE 3. Effect of 10 µmols/kg/day of chlorinated diphenyl ethers for 3 days po on NADPH cytochrome c reductase. Asterisk indicates a result significantly different from control ($P<0.05$). Average control value is 131 nmols cytochrome c reduced/min/mg protein.

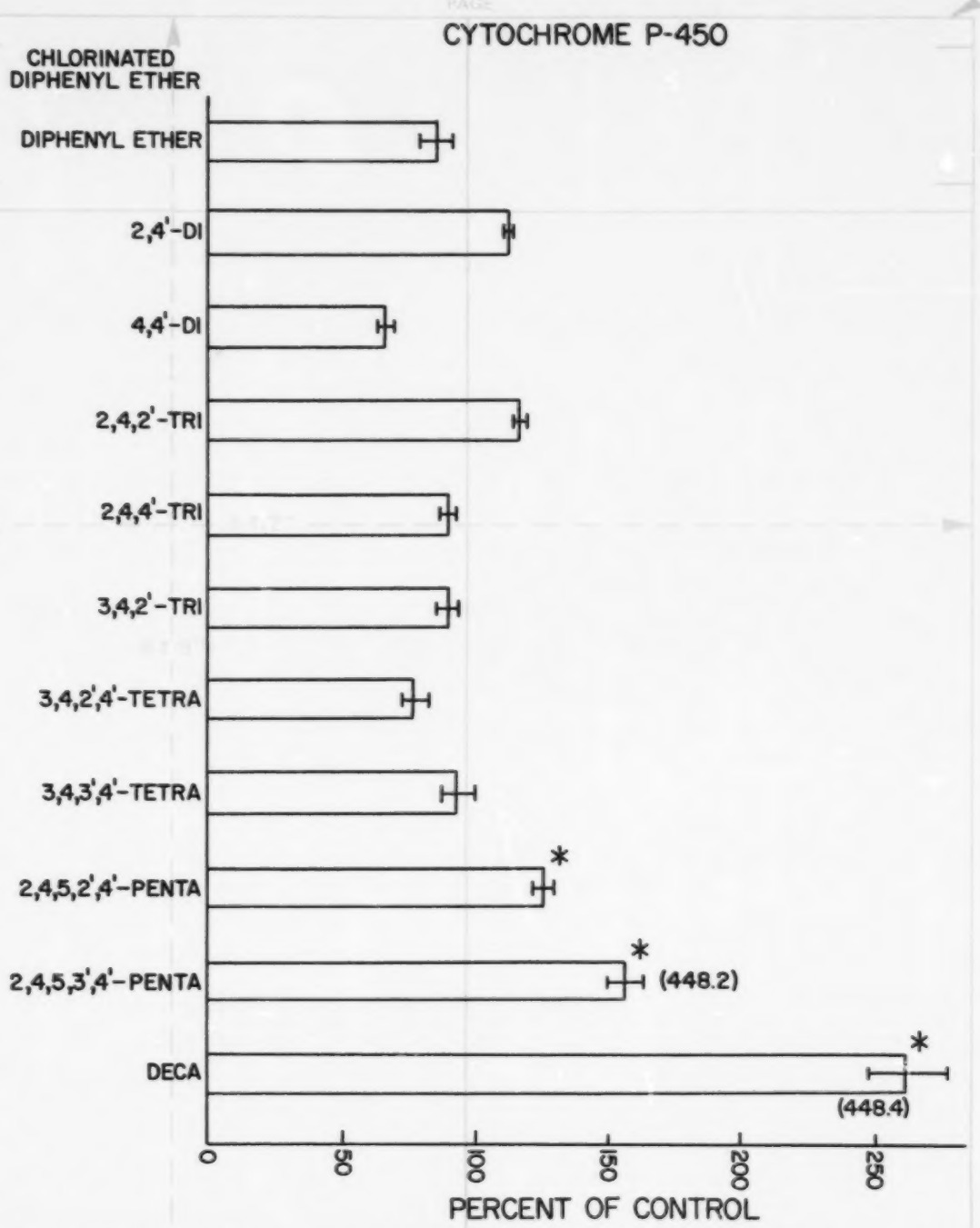

FIGURE 4. Effect of 10 µmols/kg/day of chlorinated diphenyl ethers for 3 days po on cytochrome P-450. Asterisk indicates a result significantly different from control ($P<0.05$). Average control value is 0.90 nmol/mg protein.

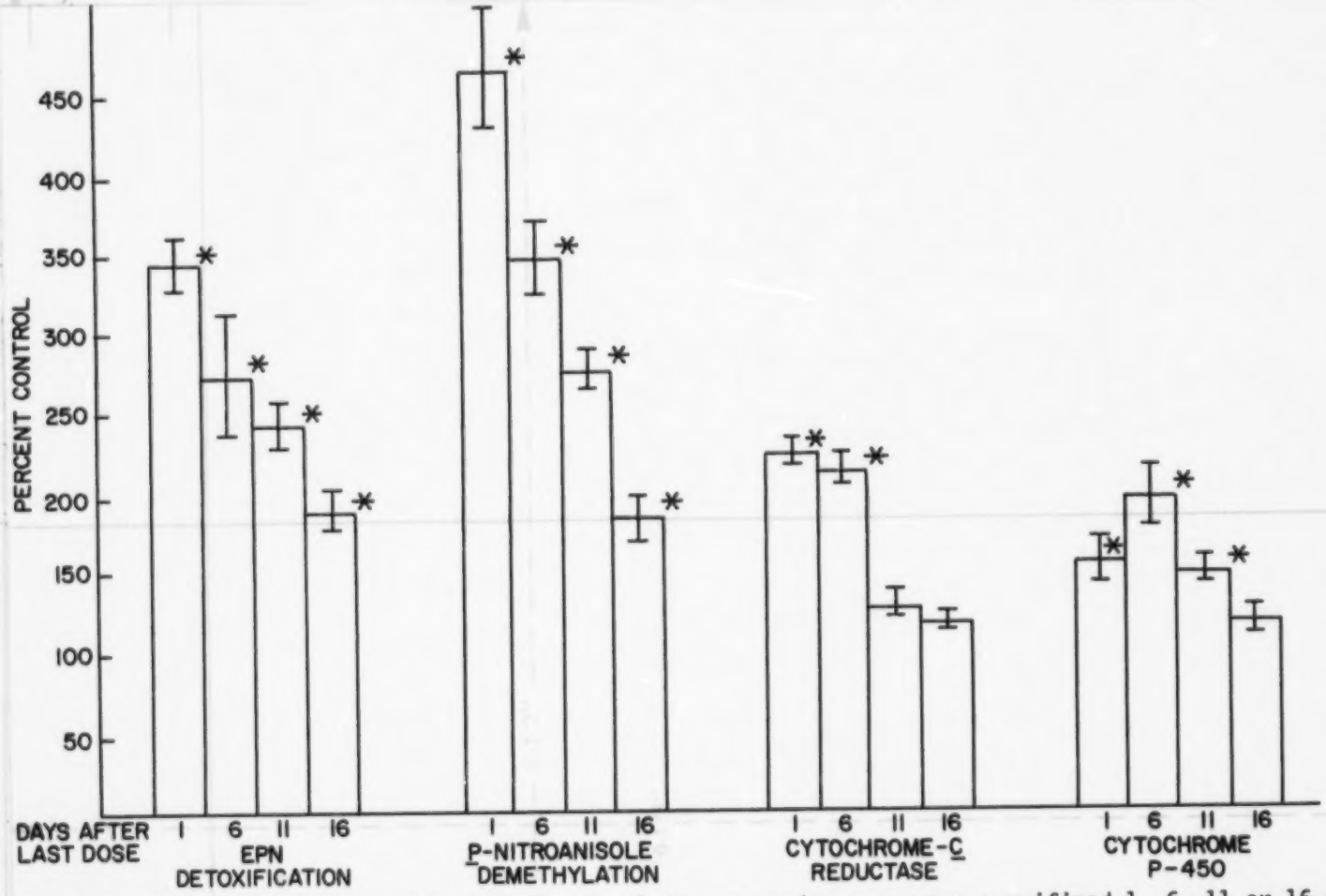

FIGURE 5. Rats received 1 mmol/kg/day TBB for 7 days po and groups were sacrificed 1, 6, 11 or 16 days later for measurements of xenobiotic metabolism. Asterisk indicates values significantly different from controls ($P<0.05$). Average control values: EPN detoxification, 5.9 ± 0.2 µg p-nitrophenol/50 mg/30 min; p-nitroanisole demethylation, 5.1 ± 0.3 µg p-nitrophenol/50 mg/30 min; NADPH-cytochrome c reductase, 118 ± 6 nmol cytochrome c reduced/min/mg protein; P-450, 0.85 ± 0.07 nmol/mg protein.

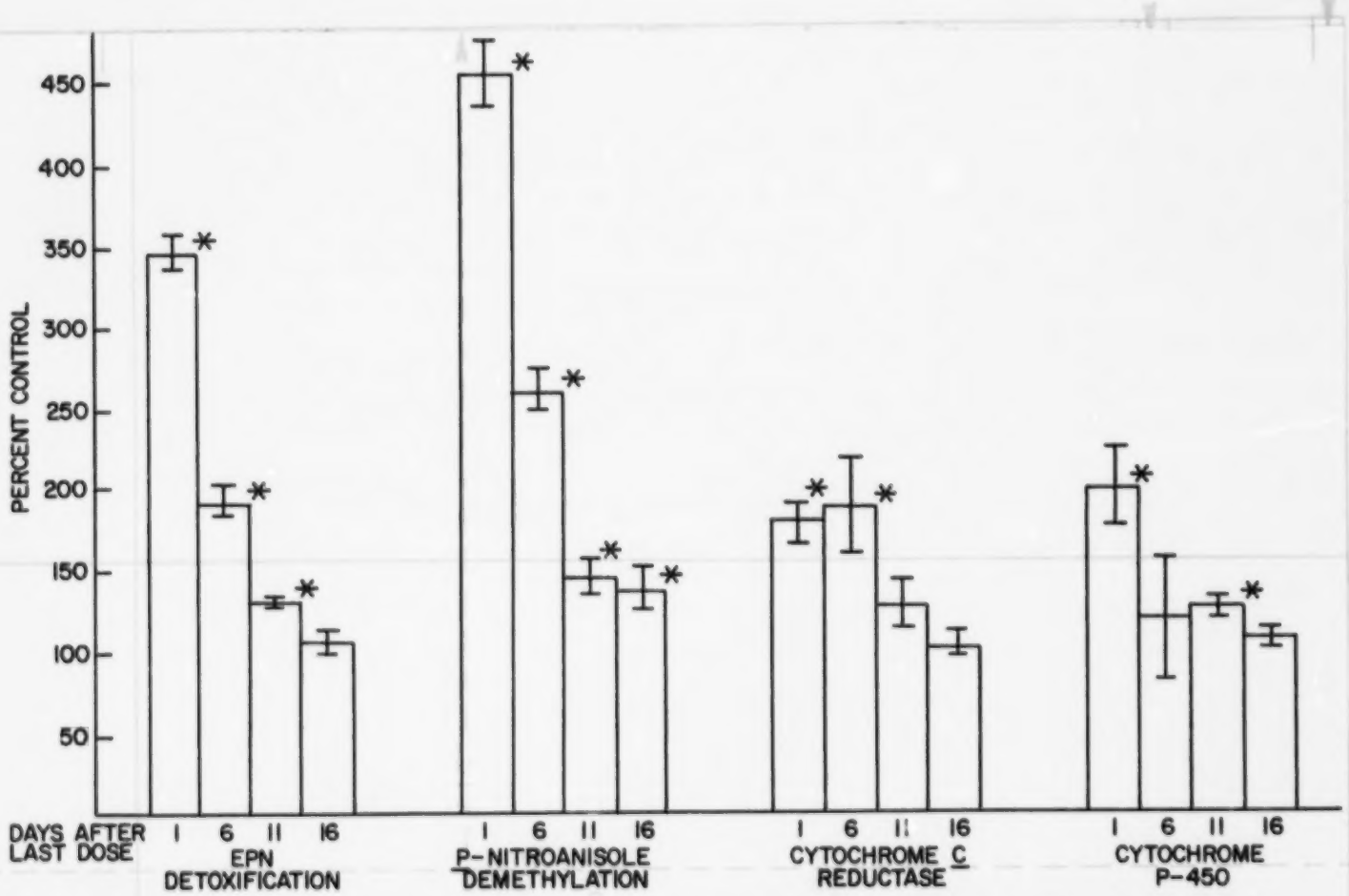

FIGURE 6. Experimental details are given in Figure 5. Average control values: EPN detoxification, 6.3 ± 0.4 µg/50 mg/30 min; p-nitroanisole demethylation, 5.1 ± 0.2 µg/50 mg/30 min; NADPH-cytochrome c reductase, 118 ± 6 nmol/min/mg; P-450, 0.85 ± 0.07 nmol/mg.

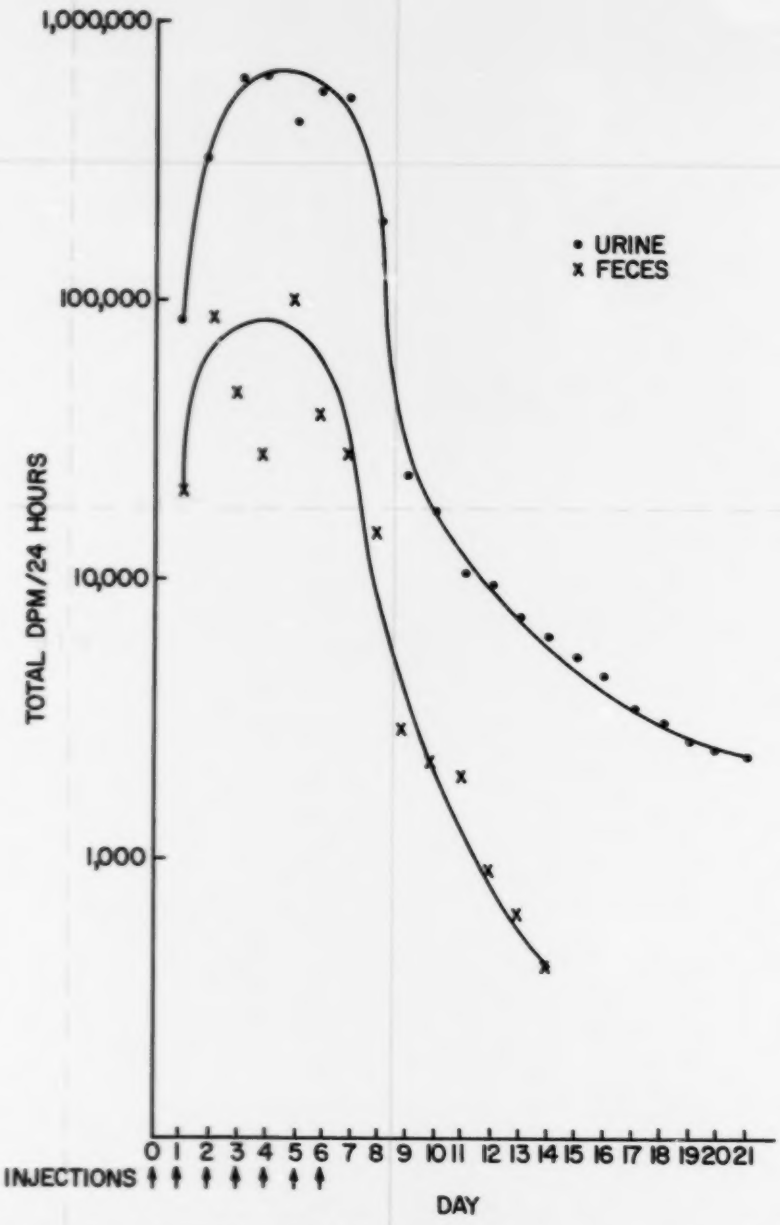

FIGURE 7. Urinary and fecal excretion of ^{14}C during and after 7 days of administration of [^{14}C]TBB. Groups of 4 rats were dosed on days indicated by arrows. Values are for 24-hour collections (first sample at 18 hours). A total of approximately 3 µCi (6,660,000 dpm) was administered per rat; 3,789,000 dpm was recovered in urine and feces.

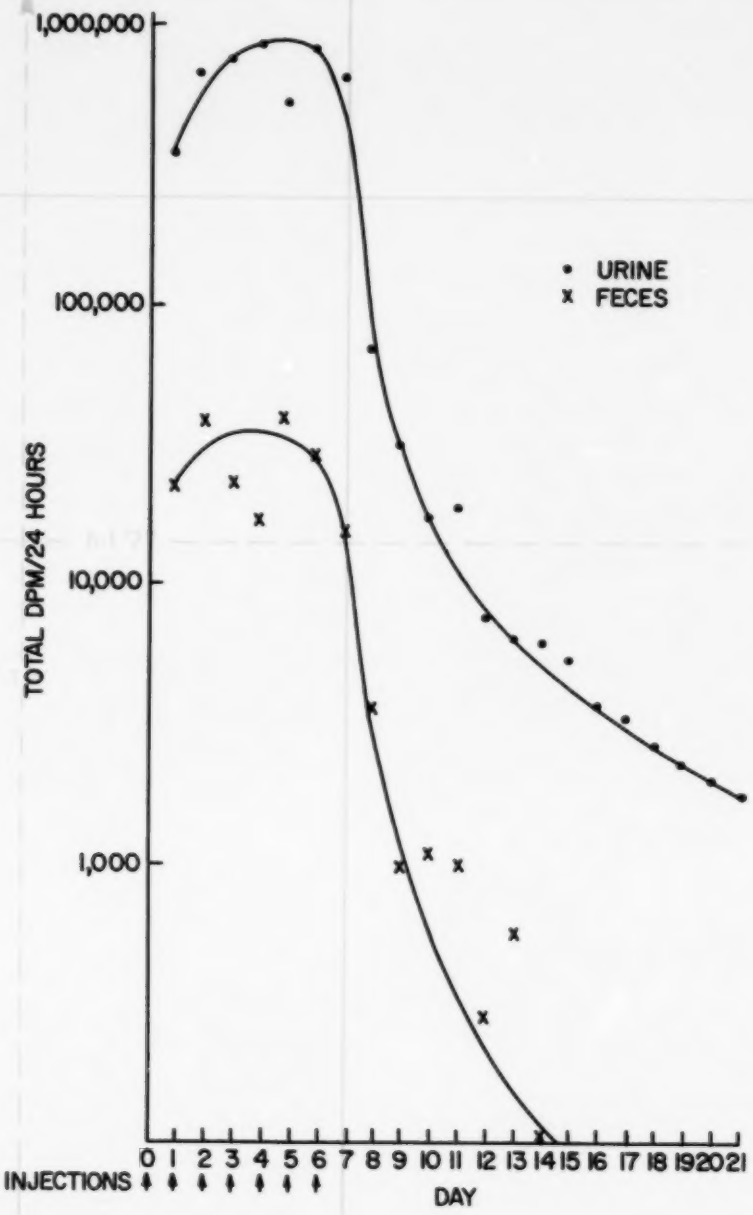

FIGURE 8. Urinary and fecal excretion of ^{14}C during and after 7 days of administration of [^{14}C]TCB. Groups of 4 rats were dosed on days indicated by arrows. Values are for 24-hour collections (first sample at 18 hours). A total of approximately 3 µCi (6,660,000 dpm) was administered per rat; 5,051,000 dpm was recovered in urine and feces.

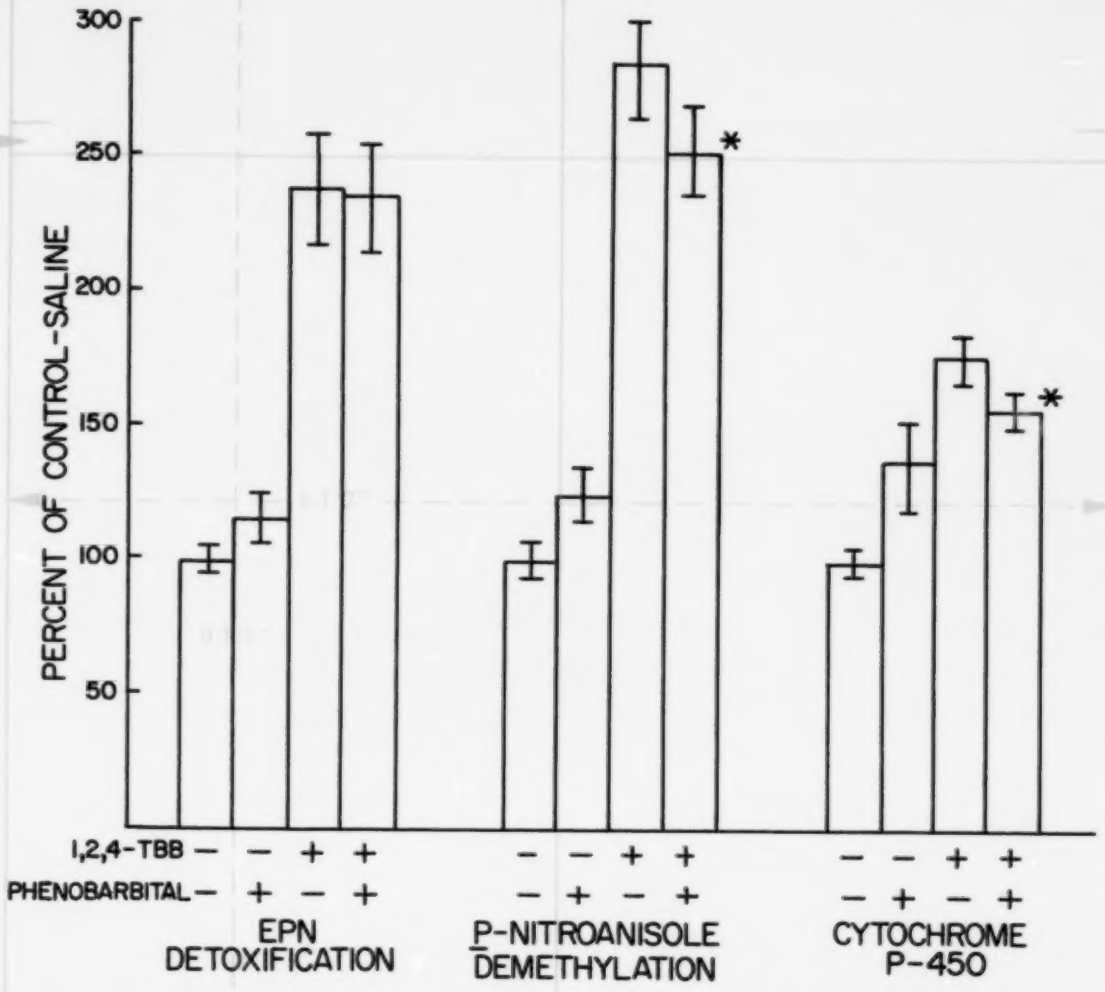

FIGURE 9. Effect of phenobarbital treatment on EPN detoxification, p-nitroanisole demethylation, and cytochrome P-450 in livers from rats receiving TBB. Some received phenobarbital (80 mg/kg/day ip for 4 days) followed by 6 days of recovery. Asterisks denote significant interaction between TBB and phenobarbital (two-way ANOVA, $P<0.05$). A significant TBB effect was also seen for all three measurements. Average control values: EPN detoxification, 6.9 ± 0.4 µg p-nitrophenol/50 mg/30 min; p-nitroanisole demethylation, 6.2 ± 0.5 µg p-nitrophenol/50 mg/30 min; P-450, 1.19 ± 0.07 nmol/mg protein.

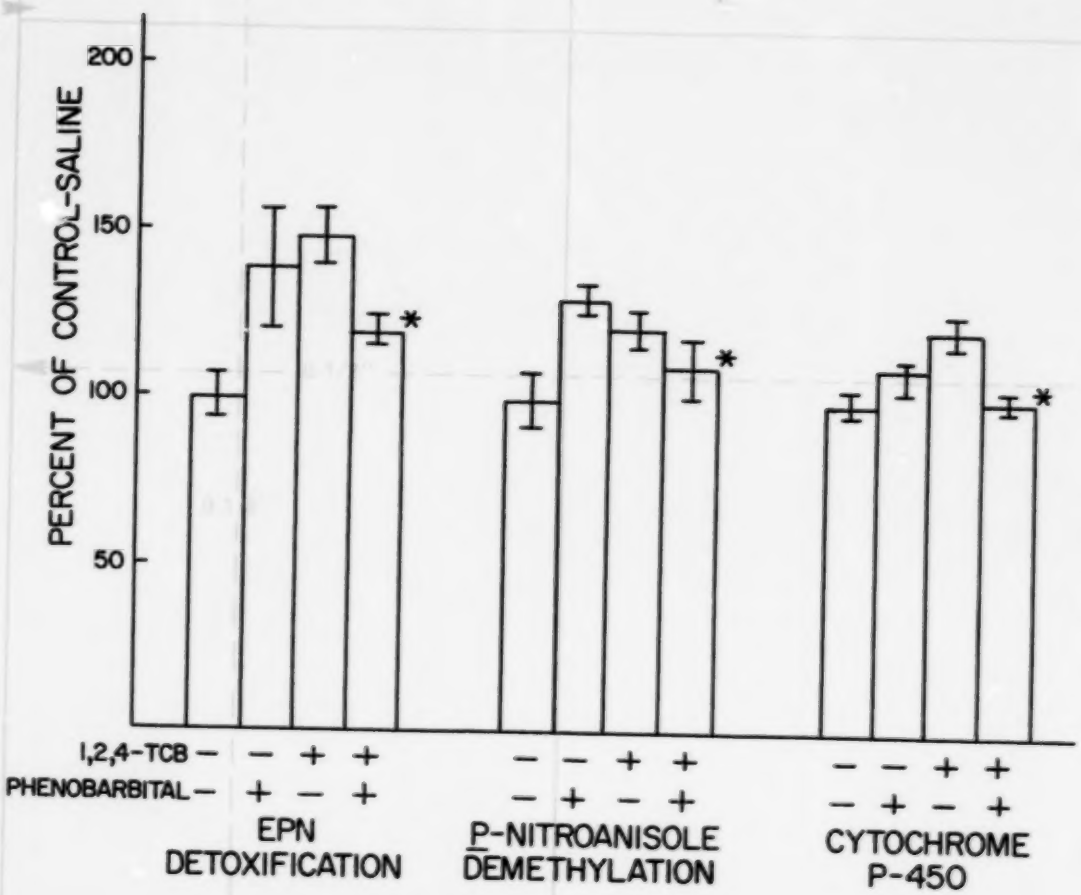

FIGURE 10. Effect of phenobarbital treatment on EPN detoxification, p-nitroanisole demethylation, and cytochrome P-450 in livers from rats receiving TCB. Details are as in Figure 9 legend. A significant TCB effect was seen for EPN detoxification and P-450. Average control values: EPN detoxification, 6.7 ± 0.5 µg/50 mg/30 min; p-nitroanisole demethylation, 6.7 ± 0.6 µg/50 mg/30 min; P-450, 1.17 ± 0.05 nmol/mg protein.

TABLE 1. EFFECT OF HEXABROMOBENZENE PO ON PORPHYRIN PRODUCTION AND EXCRETION IN FEMALE RATS

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porphyrins (µg/24 h)
		30 days of administration	
0	7.56 ± 0.42[a]	305 ± 13[a]	1.95 ± 0.07[a]
50	8.23 ± 0.14[a]	364 ± 12[b]	1.35 ± 0.08[b]
100	8.16 ± 0.19[a]	382 ± 25[b]	1.99 ± 0.28[a]
200	7.36 ± 0.24[a]	348 ± 11[a,b]	1.50 ± 0.13[b]
		60 days of administration	
0	6.98 ± 0.18[a]	314 ± 36[a]	1.10 ± 0.18[a]
50	7.28 ± 0.31[a]	662 ± 28[b]	1.08 ± 0.11[a]
100	7.38 ± 0.43[a]	535 ± 38[b]	0.87 ± 0.07[a]
200	7.36 ± 0.19[a]	624 ± 78[b]	0.92 ± 0.14[a]
		90 days of administration	
0	7.43 ± 0.33[a,b]	574 ± 20[a]	1.97 ± 0.43[a]
50	6.85 ± 0.20[a]	815 ± 108[b]	2.00 ± 0.23[a]
100	7.80 ± 0.23[b]	801 ± 61[b]	1.78 ± 0.22[a]
200	7.32 ± 0.20[a,b]	798 ± 81[b]	2.81 ± 0.40[a]
		120 days of administration	
0	7.95 ± 0.38[a]	497 ± 26[a]	2.08 ± 0.30[a]
50	7.21 ± 0.25[a,b]	552 ± 36[a]	2.01 ± 0.35[a]
100	8.00 ± 0.34[a,b]	694 ± 51[b]	1.76 ± 0.27[a]
200	8.78 ± 0.52[b]	705 ± 47[b]	2.19 ± 0.48[a]

[a,b]Values with same superscript are not significantly different (P>0.05).

TABLE 2. EFFECT OF 1,4-DIBROMOBENZENE PO ON PORPHYRIN PRODUCTION AND EXCRETION IN FEMALE RATS

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porphyrins (µg/24 h)
	30 days of administration		
0	7.72 ± 0.54[a]	437 ± 46[a]	1.48 ± 0.11[a]
50	7.30 ± 0.28[a]	506 ± 46[a]	1.32 ± 0.17[a]
100	8.36 ± 0.20[a,b]	531 ± 54[a]	1.81 ± 0.04[a]
200	9.34 ± 0.31[b]	574 ± 59[a]	1.75 ± 0.39[a]
	60 days of administration		
0	7.37 ± 0.33[a]	416 ± 46[a]	1.89 ± 0.31[a]
50	8.11 ± 0.14[a,b]	467 ± 33[a]	1.36 ± 0.20[a,b]
100	8.97 ± 0.15[b,c]	482 ± 55[a]	1.12 ± 0.08[b]
200	9.30 ± 0.49[c]	534 ± 37[a]	1.60 ± 0.25[a,b]
	90 days of administration		
0	6.58 ± 0.38[a]	295 ± 12[a]	1.33 ± 0.17[a]
50	8.02 ± 0.36[b]	405 ± 9[b]	1.13 ± 0.18[a]
100	9.81 ± 0.22[c]	445 ± 20[b]	1.65 ± 0.48[a]
200	10.03 ± 0.38[c]	603 ± 32[c]	1.40 ± 0.14[a]
	120 days of administration		
0	7.98 ± 0.38[a]	301 ± 24[a]	2.08 ± 0.30[a]
50	8.97 ± 0.45[a,b]	417 ± 19[b]	2.01 ± 0.35[a]
100	10.29 ± 0.27[b,c]	436 ± 10[b]	1.76 ± 0.27[a]
200	11.32 ± 0.67[c]	518 ± 57[b]	2.19 ± 0.48[a]

[a-c]Values with same superscript are not significantly different (P>0.05).

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 2417)
TYPING GUIDE SHEET

TABLE 3. EFFECT OF 1,2,4-TRIBROMOBENZENE PO ON PORPHYRIN PRODUCTION AND EXCRETION IN FEMALE RATS

Dose (mg/kg)	Liver wt (g)	Liver porphyrins (ng/g)	Urine porphyrins (µg/24 h)
	30 days of administration		
0	6.46 ± 0.34[a]	526 ± 49[a]	2.19 ± 0.28[a]
50	8.94 ± 0.56[b]	602 ± 90[a]	2.29 ± 0.69[a]
100	8.94 ± 0.56[b]	620 ± 66[a]	2.40 ± 0.13[a]
200	9.52 ± 0.30[b]	471 ± 22[a]	2.53 ± 0.27[a]
	60 days of administration		
0	7.30 ± 0.21[a]	156 ± 64[a]	1.92 ± 0.58[a]
50	9.16 ± 0.42[b]	251 ± 75[a]	1.61 ± 0.21[a]
100	9.96 ± 0.37[b]	285 ± 78[a]	1.77 ± 0.48[a]
200	9.72 ± 0.50[b]	285 ± 54[a]	2.42 ± 0.54[a]
	90 days of administration		
0	6.44 ± 0.26[a]	468 ± 12[a]	1.41 ± 0.23[a,b]
50	8.97 ± 0.43[b]	629 ± 20[b]	1.17 ± 0.18[a]
100	10.07 ± 0.18[b]	700 ± 17[c]	2.43 ± 0.59[b]
200	12.20 ± 0.88[c]	711 ± 26[c]	1.88 ± 0.35[a,b]
	120 days of administration		
0	7.27 ± 0.38[a]	411 ± 13[a]	1.75 ± 0.39[a]
50	11.11 ± 0.80[b]	560 ± 33[b]	1.81 ± 0.42[a]
100	12.71 ± 0.84[b]	646 ± 28[b,c]	2.27 ± 0.56[a]
200	15.18 ± 0.23[c]	682 ± 45[c]	2.13 ± 0.40[a]

[a-c]Values with same superscript are not significantly different (P>0.05).

TABLE 4. EFFECT OF TRICHLOROPHENOLS ON EPN DETOXIFICATION, CYTOCHROME c REDUCTASE AND CYTOCHROME P-450

Compound	Dose (mg/kg/day)	EPN detoxification (μg p-nitrophenol/ 50 mg/30 min)	Cytochrome c reductase (nmol cyto. c reduced/min/ mg protein	Cytochrome P-450 (nmol/mg protein)
2,3,5-Trichlorophenol	0	8.2 ± 0.7[a]	186 ± 7[a]	1.03 ± 0.06[a]
	25	9.8 ± 0.9[a]	184 ± 11[a]	0.91 ± 0.06[a]
	100	8.8 ± 0.4[a]	181 ± 11[a]	0.98 ± 0.02[a]
	400	8.2 ± 0.6[a]	157 ± 4[a]	0.91 ± 0.03[a]
2,3,6-Trichlorophenol	0	8.4 ± 1.0[a]	147 ± 8[a]	0.90 ± 0.10[a]
	25	8.4 ± 0.7[a]	146 ± 13[a]	0.92 ± 0.11[a]
	100	7.7 ± 0.6[a]	167 ± 16[a]	1.12 ± 0.08[a]
	200	7.5 ± 0.2[a]	184 ± 18[a]	0.96 ± 0.09[a]
2,4,5-Trichlorophenol	0	5.2 ± 1.8[a]	205 ± 17[a]	1.12 ± 0.09[a]
	25	7.7 ± 0.5[a]	168 ± 8[b]	1.09 ± 0.04[a]
	100	5.6 ± 0.4[a]	175 ± 9[a,b]	0.96 ± 0.05[a,b]
	400	7.1 ± 1.3[a]	105 ± 5[c]	0.84 ± 0.03[b]
2,4,6-Trichlorophenol	0	6.0 ± 0.2[a,b]	238 ± 34[a]	1.40 ± 0.16[a]
	25	6.6 ± 0.5[b]	241 ± 23[a]	1.36 ± 0.19[a]
	100	5.0 ± 0.3[a]	252 ± 23[a]	1.17 ± 0.09[a]
	400	6.0 ± 0.4[a,b]	270 ± 14[a]	1.52 ± 0.15[a]

[a-c]For each compound, values with same superscript are not significantly different from one another (P>0.05). Compounds were administered over 14 days po.

TABLE 5. EFFECT OF TRICHLOROPHENOLS IN VITRO ON p-NITROANISOLE O-DEMETHYLATION AND EPN DETOXIFICATION

Treatment	p-Nitroanisole O-demethylation[c] (µg p-nitrophenol/ 50 mg/30 min)	EPN detoxification[c] (µg p-nitrophenol/ 50 mg/30 min)
Control [a]	6.64 ± 0.68	8.39 ± 0.34
2,3,5-Trichlorophenol[b]	0.57 ± 0.21[d]	4.02 ± 0.49[d]
2,3,6-Trichlorophenol[b]	2.02 ± 0.22[d]	4.58 ± 1.14[d]
2,4,5-Trichlorophenol[b]	0.61 ± 0.24[d]	3.88 ± 0.47[d]
2,4,6-Trichlorophenol[b]	2.33 ± 0.19[d]	4.03 ± 1.17[d]

[a] 50 µl benzene added.

[b] Compound added in 50 µl benzene to give final concentration of 2.5×10^{-4} M.

[c] Mean ± S.E. of 4 experiments.

[d] Significantly different from control ($P<0.05$).

TABLE 6. EFFECT OF TRICHLOROPHENOLS ON GLUCURONYLTRANSFERASE

Compound[a]	nmol naphthol/min/mg protein
Control	6.16 ± 0.63[b]
2,3,5-Trichlorophenol	4.26 ± 0.28
2,3,6-Trichlorophenol	5.48 ± 0.72
2,4,5-Trichlorophenol	4.53 ± 0.76
2,4,6-Trichlorophenol	5.57 ± 1.01

[a] Compound was administered at a dose of 200 mg/kg daily for 14 days po. Controls received corn oil. 5 rats/groups.

[b] Mean ± S.E. No values were significantly different from control.

TABLE 7. EFFECT OF TRICHLOROPHENOLS IN VITRO ON GLUCURONYLTRANSFERASE

Treatment	nmol naphthol/min/mg protein
Control[a]	2.81 ± 0.20
2,3,5-Trichlorophenol[b]	2.06 ± 0.18[d]
2,3,6-Trichlorophenol[b]	2.27 ± 0.49
2,4,5-Trichlorophenol[b]	2.16 ± 0.11[d]
2,4,6-Trichlorophenol[b]	2.31 ± 0.81

[a]50 µl benzene added.

[b]Compound added in 50 µl benzene to give final concentration of 1×10^{-4} M.

[c]Mean ± S.E. of 4 experiments.

[d]Significantly different from control ($P<0.05$).

TABLE 8. HEPATOTOXICITY OF THE TRICHLOROPHENOLS

Compound[a]	Glucose-6-phosphatase (µmols PO_4/g/min)	Sorbitol dehydrogenase (µmols/ml serum/hr)
Control	19.1 ± 2.2[b]	0.25 ± 0.11[b]
2,3,5-Trichlorophenol	19.5 ± 0.8	0.08 ± 0.04
2,3,6-Trichlorophenol	20.8 ± 0.8	0.22 ± 0.05
2,4,5-Trichlorophenol	21.1 ± 0.7	0.16 ± 0.05
2,4,6-Trichlorophenol	17.8 ± 0.9	0.06 ± 0.03

[a]Compound was administered at a dose of 200 mg/kg daily for 14 days po. Controls received corn oil. 4 rats/group.

[b]Mean ± S.E. No values were significantly different from control.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA Form 2250-4 (4-80)
(PREVIOUSLY CIN: EPA FORM 2511)
TYPING GUIDE SHEET

TABLE 9. COMPOSITION OF BROMODIPHENYL ETHERS

Compound	Percent of bromodiphenyl ether									mol. wt. or conglomerate mol. wt.
	0	2	4	5	6	7	8	9	10	
Diphenyl ether	98									170
Bis(p-bromophenyl)ether		a								328
Pentabromodiphenyl ether			24.6	58.1	13.3	2.6	0.3	0.2	0.8	564
Octabromodiphenyl ether				1.1	8.5	45.1	30.7	13.0	1.6	766
Decabromodiphenyl ether									b	959

[a]Reagent grade.

[b]High purity.

TABLE 10. EFFECT OF BROMODIPHENYL ETHERS ON LIVER WT./BODY WT., NADPH CYTOCHROME c REDUCTASE, AND CYTOCHROME P-450

Treatment[a]	$\frac{\text{Liver wt.}}{\text{Body wt.}} \cdot 100$[b]	Cytochrome c reductase, nmol cyto. c reduced/min/mg protein[b]	Cytochrome P-450 nmol/mg protein[b]
Control	3.80 ± 0.12[c]	151 ± 26[c]	0.79 ± 0.05[c]
Diphenyl ether	4.03 ± 0.13[c,d]	134 ± 25[c]	0.71 ± 0.04[c]
Bis(p-bromophenyl) ether	4.48 ± 0.12[d,e]	238 ± 13[d,e]	1.61 ± 0.09[d]
Pentabromodiphenyl ether	6.25 ± 0.25[f]	315 ± 20[f]	2.56 ± 0.17[e]
Octabromodiphenyl ether	5.54 ± 0.25[g]	281 ± 34[e,f]	1.86 ± 0.14[d]
Decabromodiphenyl ether	4.75 ± 0.10[e]	175 ± 5[c,d]	0.91 ± 0.05[c]

[a]0.1 mmol/kg/day for 14 days po. Controls received corn oil.

[b]Mean ± S.E. of 4 rats.

[c-g]Values with the same superscript are not significantly different from one another (P>0.05).

TABLE 11. EFFECT OF BROMODIPHENYL ETHERS ON EPN DETOXIFICATION, p-NITROANISOLE DEMETHYLATION, BENZO[A]PYRENE HYDROXYLASE, AND UDP-GLUCURONYLTRANSFERASE

Treatment[a]	EPN detoxification, µg p-nitrophenol/ 50 mg/30 min[b]	p-Nitroanisole demethylation, µg p-nitrophenol/ 50 mg/30 min[b]	UDP-Glucuronyl-transferase, nmol naphthol/ mg protein/min[b]	Benzo[a]pyrene hydroxylase, nmol/mg protein/10 min[b]
Control	6.60 ± 1.12[c]	6.33 ± 0.78[c,d]	6.23 ± 1.25[c]	6.79 ± 0.54[c]
Diphenyl ether	11.08 ± 1.72[d]	5.68 ± 0.85[c]	5.76 ± 0.73[c]	4.44 ± 0.55[c]
Bis(p-bromophenyl) ether	10.42 ± 1.24[c,d]	9.25 ± 0.75[d]	7.65 ± 1.12[c,d]	4.86 ± 0.74[c]
Pentabromodiphenyl ether	15.98 ± 1.22[e]	43.81 ± 1.93[e]	10.87 ± 1.74[d]	11.01 ± 1.07[d]
Octabromodiphenyl ether	16.30 ± 1.17[e]	17.84 ± 0.41[f]	10.36 ± 1.14[d]	6.01 ± 1.06[c]
Decabromodiphenyl ether	7.10 ± 0.85[c]	8.68 ± 1.06[c,d]	5.97 ± 0.98[c]	5.52 ± 0.61[c]

[a] 0.1 mmol/kg/day for 14 days po. Controls received corn oil.

[b] Mean ± S.E. of 4 rats except: 3 rats in pentabromodiphenyl ether group in liver wt./body wt. and glucuronyltransferase and 2 for that group in benzo[a]pyrene hydroxylase.

[c-f] Values with the same superscript are not significantly different from one another (P>0.05).

TABLE 12. EFFECT OF PENTABROMODIPHENYL ETHER OR OCTABROMODIPHENYL ETHER ON EPN DETOXIFICATION, p-NITROANISOLE DEMETHYLATION, CYTOCHROME c REDUCTASE ACTIVITY, AND CYTOCHROME P-450 CONTENT

Dose µmol/kg/day for 90 days	EPN detoxification, µg p-nitrophenol/ 50 mg/30 min	p-Nitroanisole demethylation, µg p-nitrophenol/ 50 mg/30 min	Cytochrome c reductase, nmol cyto. c reduced/ min/mg protein	Cytochrome P-450, nmol/mg protein
		Pentabromodiphenyl ether		
0	9.6 ± 0.3[a]	7.6 ± 0.1[a]	88 ± 6[a]	0.60 ± 0.04[a]
6.25	17.7 ± 0.5[b]	25.6 ± 1.2[b]	117 ± 9[b]	1.02 ± 0.06[b]
12.5	21.5 ± 0.6[c]	34.6 ± 1.1[c]	120 ± 5[b]	1.16 ± 0.07[b]
25	26.5 ± 0.7[d]	50.1 ± 1.3[d]	138 ± 7[b]	1.14 ± 0.07[b]
		Octabromodiphenyl ether		
0	9.7 ± 0.5[a]	10.0 ± 0.4[a]	171 ± 11[a]	1.04 ± 0.10[a]
6.25	21.3 ± 1.1[b]	20.9 ± 1.8[b]	197 ± 9[a]	1.56 ± 0.13[b]
12.5	26.9 ± 2.2[b]	27.3 ± 1.3[c]	196 ± 18[a]	1.27 ± 0.16[a,b]
25	37.1 ± 2.0[c]	38.4 ± 1.9[d]	208 ± 11[a]	1.46 ± 0.09[b]

[a-d]Values with same superscript are not significantly different from one another (P>0.05).

TABLE 13. EFFECT OF PENTABROMODIPHENYL ETHER ON EPN DETOXIFICATION, p-NITROANISOLE DEMETHYLATION, NADPH CYTOCHROME c REDUCTASE, AND CYTOCHROME P-450

Dose, µmol/kg/day for 90 days	EPN detoxification, µg p-nitrophenol/ 50 mg/30 min	p-Nitroanisole demethylation, µg p-nitrophenol/ 50 mg/30 min	NADPH Cytochrome c reductase, nmol cyto. c reduced/ min/mg protein	Cytochrome P-450, nmol/mg protein
		Administration		
0	5.8 ± 0.1[a]	5.5 ± 0.4[a]	165 ± 10[a]	1.05 ± 0.02[a]
0.78	8.1 ± 0.2[b]	9.7 ± 0.6[b]	215 ± 13[b]	1.41 ± 0.07[b]
1.56	8.9 ± 0.3[b]	12.2 ± 0.8[c]	194 ± 21[a,b]	1.26 ± 0.06[b]
3.13	11.4 ± 0.6[c]	16.9 ± 0.4[d]	209 ± 14[a,b]	1.25 ± 0.09[b]
		Administration plus a 30-day recovery period		
0	6.0 ± 0.2[a]	5.5 ± 0.4[a]	128 ± 9[a]	0.80 ± 0.07[a]
0.78	7.2 ± 0.2[b]	7.5 ± 0.3[b]	138 ± 1[a,b]	0.87 ± 0.04[a]
1.56	7.4 ± 0.5[b]	7.9 ± 0.4[b]	138 ± 8[a,b]	0.96 ± 0.18[a]
3.13	9.1 ± 0.2[c]	10.7 ± 0.9[c]	152 ± 6[b]	0.90 ± 0.13[a]
		Administration plus a 60-day recovery period		
0	6.3 ± 0.3[a]	3.6 ± 0.3[a]	155 ± 9[a]	0.82 ± 0.07[a]
0.78	6.4 ± 0.3[a]	3.8 ± 0.2[a]	162 ± 14[a]	0.85 ± 0.12[a]
1.56	7.7 ± 0.3[b]	4.6 ± 1.0[b]	148 ± 7[a]	0.85 ± 0.06[a]
3.13	7.5 ± 0.4[b]	5.6 ± 0.3[c]	172 ± 8[a]	0.82 ± 0.06[a]

[a-d]For each time period, values with same superscript are not significantly different from one another (P>0.05).

TABLE 14. EFFECT OF OCTABROMODIPHENYL ETHER ON EPN DETOXIFICATION, p-NITROANISOLE DEMETHYLATION, NADPH CYTOCHROME c REDUCTASE, AND CYTOCHROME P-450

Dose, µmol/kg/day for 90 days	EPN detoxification, µg p-nitrophenol/ 50 mg/30 min	p-Nitroanisole demethylation, µg p-nitrophenol/ 50 mg/30 min	NADPH Cytochrome c reductase, nmol cyto. c reduced/ min/mg protein	Cytochrome P-450, nmol/mg protein
		Administration		
0	7.1 ± 0.3[a]	9.0 ± 0.4[a]	199 ± 14[a]	1.08 ± 0.09[a]
0.78	9.5 ± 0.4[b]	11.9 ± 0.6[b]	190 ± 8[a]	1.04 ± 0.05[a]
1.56	11.0 ± 0.6[b]	13.8 ± 0.4[c]	217 ± 13[a]	1.21 ± 0.06[a]
3.13	12.4 ± 0.5[c]	14.5 ± 0.8[c]	216 ± 22[a]	1.24 ± 0.08[a]
		Administration plus a 30-day recovery period		
0	10.6 ± 0.4[a,b]	12.0 ± 0.6[a]	104 ± 4[a,b]	0.88 ± 0.05[a]
0.78	9.9 ± 0.9[a]	13.4 ± 0.8[a,b]	86 ± 6[a]	0.82 ± 0.05[a]
1.56	10.9 ± 0.8[a,b]	13.7 ± 0.4[a,b]	109 ± 8[b]	0.89 ± 0.05[a]
3.13	12.4 ± 0.7[b]	14.3 ± 0.7[b]	103 ± 8[a,b]	0.88 ± 0.03[a]
		Administration plus a 60-day recovery period		
0	6.8 ± 0.3[a]	6.3 ± 0.3[a]	157 ± 6[a]	0.93 ± 0.02[a]
0.78	8.3 ± 0.4[a,b]	7.9 ± 0.3[b]	151 ± 5[a]	0.95 ± 0.06[a]
1.56	9.2 ± 0.9[b]	8.4 ± 0.3[b]	165 ± 6[a]	0.92 ± 0.02[a]
3.13	11.4 ± 0.7[c]	10.6 ± 0.7[c]	165 ± 7[a]	0.92 ± 0.04[a]

[a-c]For each time period, values with same superscript are not significantly different from one another (P>0.05).

TABLE 15. COMPARISON OF ORAL VERSUS INTRAPERITONEAL ROUTES OF ADMINISTRATION OF 2,5-DICHLORO-p-BENZOQUINONE

Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	No. dying / No. started
po																															
20 mg/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2/5
100 mg/kg	0	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4/5
ip																															
20 mg/kg	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1[a]	0	0	0	0	0	0	0	0	0	0	0	0	4/5[a]
100 mg/kg	0	5																													5/5

[a]Fifth animal was sacrificed.

TABLE 16. EFFECT OF BENZOQUINONE AND DERIVATIVES ON METHEMOGLOBIN FORMATION AND SORBITOL DEHYDROGENASE

Compound[a]	N[b]	Methemoglobin, percent	Sorbitol dehydrogenase, µmols/ml serum/hr
Control[a]	4	4.23 ± 0.47	0.14 ± 0.03
p-Benzoquinone	4	5.99 ± 1.53	0.15 ± 0.05
2,5-Dichloro-p-benzoquinone	2	3.28 ± 0.34	0.11 ± 0.00
2,6-Dichloro-p-benzoquinone	3	2.98 ± 1.54	0.09 ± 0.05
2,5-Dimethyl-p-benzoquinone	3	4.75 ± 0.34	0.27 ± 0.03

[a]Compound was administered at a dose of 12.5 mg/kg/day for 14 days po.

[b]Each group started with 4 rats except for the p-benzoquinone group which had 5 animals.

TABLE 17. EFFECT OF BENZOQUINONES ON THE MICROSOMAL SYSTEM FOR METABOLISM OF FOREIGN ORGANIC COMPOUNDS[a]

Dose, mg/kg/day	Number started	Number dying	Final body weight, g	EPN detoxification, µg/p-nitrophenol/ 50 mg/30 min	Cytochrome c reductase, nmol cyto. c reduced/min/mg protein	Cytochrome P-450, nmol/mg protein
			Effect of 2,6-dichloro-p-benzoquinone			
0	6	0	278 ± 7	7.4 ± 0.2	223 ± 16	1.27 ± 0.23
25	6	4	262 ± 22	6.5 ± 0.4	249 ± 141	1.07 ± 0.41
100	6	4	215 ± 29	4.8 ± 0.2	201 ± 7	1.35 ± 0.09
300	6	6	-	-	-	-
			Effect of 2,5-dichloro-p-benzoquinone			
0	6	0	291 ± 7	8.1 ± 0.6	187 ± 21	1.40 ± 0.13
25	6	4	215 ± 38	7.1 ± 0.6	141 ± 17	1.00 ± 0.85
50	6	5	209	6.4	121	1.40
100	8	6	184 ± 21	3.8 ± 0.8	131 ± 36	0.81 ± 0.20
			Effect of 2,5-dimethyl-p-benzoquinone			
0	6	0	302 ± 8	7.8 ± 0.6	172 ± 11	1.11 ± 0.08
25	6	5	202	4.9	137	1.14
50	6	5	208	5.7	156	1.25
100	7	6	209	5.7	117	0.84

[a]Compound was administered for 14 days po. The parameters were mortality, body weight, EPN detoxification, and components of the microsomal system.

TABLE 18. EFFECT OF DIBENZOFURAN, 2,8-DICHLORODIBENZOFURAN, AND OCTACHLORODIBENZOFURAN ON EPN DETOXIFICATION, BENZPYRENE HYDROXYLASE, NADPH-CYTOCHROME c REDUCTASE, AND CYTOCHROME P-450

Treatment[a]	EPN detoxification, μg p-nitrophenol/ 50 mg/30 min	Benzpyrene hydroxylase, nmol/mg protein/10 min	Cytochrome c reductase, nmol cyto. c reduced/ min/mg protein	Cytochrome P-450	
				Content nmol/mg protein	Peak nm
Corn Oil	5.83 ± 0.29[b]	8.08 ± 2.06[b]	167 ± 23[b]	1.14 ± 0.17[b]	450.0 ± 0.0[b]
Dibenzofuran	6.09 ± 0.72[b]	7.17 ± 1.37[b]	151 ± 25[b]	1.09 ± 0.29[b]	450.0 ± 0.0[b]
2,8-Dichloro-dibenzofuran	7.00 ± 0.19[b]	21.49 ± 5.95[c]	142 ± 18[b]	1.55 ± 0.19[b,c]	448.2 ± 0.0[c]
Octachloro-dibenzofuran	6.90 ± 0.55[b]	13.13 ± 2.42[b,c]	161 ± 20[b]	1.85 ± 0.23[c]	448.2 ± 0.0[c]

[a]Groups of 5 rats were administered 5 μmols/kg/day of the compound for 3 days po.

[b-c]Values with same superscript are not significantly different from one another (P>0.05).

TABLE 19. CONTENT OF ^{14}C-LABELED 1,2,4-TRIBROMOBENZENE AND 1,2,4-TRICHLOROBENZENE IN TISSUES[a]

Tissue	Activity (dpm/g)[b]			
	Day 1	Day 6	Day 11	Day 16
	^{14}C-labeled 1,2,4-tribromobenzene			
Abdominal fat	9933 ± 1067	2358 ± 248	1204 ± 182	808 ± 58
Liver	2075 ± 183	675 ± 17	396 ± 19	321 ± 22
Adrenals[c]	3246 ± 1083	625 ± 56	d	
Muscle	963 ± 279	d		
Kidney	2567 ± 224	783 ± 58		
Heart	800 ± 71	d		
Spleen	808 ± 120	d		
	^{14}C-labeled 1,2,4-trichlorobenzene			
Abdominal fat	2033 ± 439	642 ± 54	342 ± 10	408 ± 39
Liver	1075 ± 87	442 ± 22	308 ± 21	317 ± 18
Adrenals[c]	754 ± 132	246 ± 22	d	
Muscle	400 ± 30	d		
Kidney	1471 ± 167	404 ± 43	d	
Heart	438 ± 14	d		
Spleen	404 ± 14	d		

[a]Rats were given TBB or TCB, 1 mmol/kg/day (= 2 µCi/kg/day) po for 7 days, followed by 1, 6, 11, or 16 days of recovery.

[b]Values are rates per gram of tissue. Each value is the mean ± S.E. for 4 rats, except 3 for abdominal fat on day 1.

[c]Total for both adrenals. They were not weighed.

[d]Value less than twice background; further analyses were not performed.

TABLE 20. INFLUENCE OF STARVATION ON EPN DETOXIFICATION, p-NITROANISOLE DEMETHYLATION, AND CYTOCHROME P-450 CONTENT OF LIVER IN RATS TREATED WITH 1,2,4-TRIBROMOBENZENE

Treatment[a]	EPN detoxification	p-Nitroanisole demethylation	Cytochrome P-450 (nmol/mg protein)
	µg p-nitrophenol/50 mg/30 min		
Control fed	6.3 ± 0.4[b]	7.1 ± 0.5[b]	0.96 ± 0.04[b]
Control starved	6.2 ± 1.4[b]	7.0 ± 0.6[b]	1.07 ± 0.05[b]
TBB fed	20.1 ± 2.4[c]	20.2 ± 1.8[c]	1.90 ± 0.09[c]
TBB starved	29.1 ± 2.0[d]	26.2 ± 1.8[d]	3.25 ± 0.12[d]

[a]Groups of 5 rats received 1 mmol/kg TBB for 7 days po, followed by 6 days of recovery and then 4 days of starvation before sacrifice. A two-way ANOVA indicated significant ($P<0.05$) drug (TBB), starvation, and interaction effects for all three assays.

[b-d]Values assigned the same superscript are not significantly different within each assay ($P<0.05$) as determined by a Newman-Keuls' analysis.

TABLE 21. INFLUENCE OF STARVATION ON EPN DETOXIFICATION, p-NITROANISOLE DEMETHYLATION, AND CYTOCHROME P-450 CONTENT OF LIVER IN RATS TREATED WITH 1,2,4-TRICHLOROBENZENE

Treatment[a]	EPN detoxification	p-Nitroanisole demethylation	Cytochrome P-450 (nmol/mg protein)
	µg p-nitrophenol/50 mg/30 min		
Control fed	6.2 ± 0.5[b]	4.6 ± 0.3[b,c]	1.14 ± 0.08[b]
Control starved	5.0 ± 0.4[b]	3.8 ± 0.5[b]	1.12 ± 0.09[b]
TCB fed	9.0 ± 0.3[c]	7.4 ± 0.6[d]	1.37 ± 0.11[b]
TCB starved	7.7 ± 0.7[c]	6.3 ± 0.8[c,d]	1.37 ± 0.13[b]

[a]Groups of 5 rats received 1 mmol/kg TCB po for 7 days followed by 6 days of recovery and then 4 days of starvation before sacrifice. A two-way ANOVA indicated a significant ($P<0.05$) drug (TCB) effect for all three assays. A significant starvation effect was seen only with EPN detoxification. No significant interaction was observed.

[b-d]Values assigned the same superscript within each assay are not significantly different ($P<0.05$) as determined by a Newman-Keuls' analysis.

TABLE 22. INFLUENCE OF STARVATION ON CONTENT OF ^{14}C IN ABDOMINAL FAT AND LIVER AFTER TCB OR TBB[a]

Group	Activity (dpm/g)	
	[^{14}C] TBB-treated	[^{14}C] TCB-treated
	Abdominal fat	
Fed	2579 ± 291[b]	103 ± 15[b]
Starved	4783 ± 403[c]	94 ± 11[b]
	Liver tissue	
Fed	286 ± 15[b]	57 ± 6[b]
Starved	558 ± 59[c]	53 ± 11[b]

[a]Groups of 4 rats received ^{14}C-labeled TCB or TBB at 1 mmol/kg/day (= 2 μCi/kg/day) po for 7 days, followed by 6 days of recovery and 4 days of food deprivation before sacrifice.

[b,c]Within each tissue group, values assigned the same superscript are not significantly different ($P<0.05$). A two-way ANOVA indicated a significant ($P<0.05$) drug, starvation, and interaction effect for both tissues.

TABLE 23. INFLUENCE OF STARVATION ON URINARY EXCRETION OF ^{14}C AFTER TCB OR TBB ADMINISTRATION

Day	Activity (dpm/24-hour urine sample)			
	1,2,4-Tribromobenzene		1,2,4-Trichlorobenzene	
	Fed	Starved	Fed	Starved
	Recovery			
5	6708 ± 297[c]	7124 ± 930[c]	2185 ± 182	2268 ± 200
6	4891 ± 349[c]	5189 ± 484[c]	1715 ± 338	1503 ± 114
	Starvation			
1	4290 ± 623[c]	6399 ± 804[d]	2704 ± 108	b
2	3060 ± 360[c]	6241 ± 1195[d]	1994 ± 208	b
3	2600 ± 175[c]	3730 ± 688[c]	b	1112 ± 156
4	2023 ± 133[c]	2789 ± 192[c]	b	b

[a]Groups of 4 rats received ^{14}C-labeled TBB or TCB at 1 mmol/kg/day (= 2 μCi/kg/day) po for 7 days, followed by 6 days of recovery and 4 days of starvation.

[b]Value less than twice background.

[c,d]Values assigned the same superscript are not significantly different ($P<0.05$) as determined by a two-way ANOVA and a Newman-Keuls' analysis of means. Comparisons were made only between the fed and starved groups on each day for the TBB-treated animals. No comparison was made between days.

TABLE 24. EFFECT OF AROCLOR 1254 ON EPN DETOXIFICATION AND p-NITROANISOLE DEMETHYLATION

Days after last dose	Treatment[a]	N	EPN detoxification, µg p-nitrophenol/ 50 mg/30 min	p-Nitroanisole demethylation, µg p-nitrophenol 50 mg/30 min
1	Control	5	10.6 ± 0.4[b]	10.5 ± 0.4[b]
	Aroclor 1254	5	38.3 ± 1.6[c]	100.8 ± 4.7[c]
11	Control	5	11.1 ± 0.5[b]	10.1 ± 0.9[b]
	Aroclor 1254	5	27.2 ± 2.6[c]	51.7 ± 7.3[c]
16	Control	5	11.0 ± 0.5[b]	6.4 ± 0.2[b]
	Control-starved	5	13.5 ± 0.7[b]	7.4 ± 0.5[b]
	Aroclor 1254	5	24.9 ± 1.3[c]	69.2 ± 3.5[c]
	Aroclor 1254-starved	5	39.4 ± 2.0[d]	65.1 ± 3.6[c]
28	Control	5	11.1 ± 0.7[b]	9.0 ± 0.3[b]
	Aroclor 1254	5	20.8 ± 0.7[c]	38.5 ± 0.9[c]
36	Control	5	8.4 ± 0.3[b]	8.8 ± 0.4[b]
	Aroclor 1254	5	18.3 ± 0.9[c]	34.3 ± 1.0[c]
49	Control	5	12.7 ± 0.4[b]	14.8 ± 1.0[b]
	Aroclor 1254	5	24.3 ± 0.8[c]	34.7 ± 2.3[c]
56	Control	5	7.6 ± 0.3[b]	5.6 ± 0.2[b]
	Control-starved	5	7.5 ± 0.2[b]	6.2 ± 0.2[b]
	Aroclor 1254	5	10.6 ± 0.4[c]	15.4 ± 1.9[c]
	Aroclor 1254-starved	5	19.7 ± 0.9[d]	25.0 ± 1.9[d]
71	Control	5	7.4 ± 0.2[b]	6.7 ± 0.5[b]
	Aroclor 1254	2	23.6[c]	51.5[c]
106	Control	5	12.2 ± 1.3[b]	5.7 ± 0.8[b]
	Control-starved	5	12.0 ± 2.0[b]	6.3 ± 0.9[b]
	Aroclor 1254	5	14.6 ± 1.3[b]	13.1 ± 2.0[c]
	Aroclor 1254-starved	5	23.8 ± 1.6[c]	18.8 ± 2.3[d]
120	Control	5	8.1 ± 0.3[b]	5.6 ± 0.7[b]
	Control-starved	5	9.2 ± 0.5[b]	6.6 ± 1.1[b]
	Aroclor 1254	5	10.7 ± 0.4[c]	7.4 ± 0.5[b]
	Aroclor 1254-starved	5	12.8 ± 0.6[c]	10.9 ± 0.7[c]
148	Control	5	7.2 ± 0.4[b]	3.2 ± 0.3[b]
	Control-starved	5	6.8 ± 0.2[b]	2.9 ± 0.7[b]
	Aroclor 1254	5	8.9 ± 0.6[c]	3.5 ± 0.5[b]
	Aroclor 1254-starved	5	8.7 ± 0.4[c]	5.6 ± 0.2[c]

[a]Groups of 5 rats were administered corn oil or Aroclor 1254, 10 mg/kg/day for 14 days po. In some cases, animals were starved for 4 days prior to sacrifice.

[b-d]Values with same superscript are not significantly different from one another (P>0.05).

TABLE 25. EFFECT OF AROCLOR 1254 on NADPH CYTOCHROME c REDUCTASE AND CYTOCHROME P-450

Days after last dose	Treatment[a]	N	Cytochrome c reductase, nmol cyto. c reduced/ min/mg protein	Cytochrome P-450, nmol/mg protein
1	Control	5	116 ± 5[b]	0.73 ± 0.03[b]
	Aroclor 1254	5	215 ± 12[c]	2.41 ± 0.17[c]
11	Control	5	192 ± 21[b]	0.96 ± 0.04[b]
	Aroclor 1254	5	166 ± 7[b]	1.63 ± 0.10[c]
16	Control	5	135 ± 14[b,c]	0.91 ± 0.11[b]
	Control-starved	5	109 ± 10[b]	0.85 ± 0.05[b]
	Aroclor 1254	5	152 ± 9[c,d]	1.56 ± 0.19[c]
	Aroclor 1254-starved	5	177 ± 12[d]	2.53 ± 0.18[d]
28	Control	5	147 ± 7[b]	0.98 ± 0.04[b]
	Aroclor 1254	5	185 ± 15[c]	1.67 ± 0.59[c]
36	Control	5	153 ± 7[b]	0.87 ± 0.03[b]
	Aroclor 1254	5	164 ± 8[b]	1.58 ± 0.13[c]
49	Control	5	138 ± 10[b]	1.02 ± 0.10[b]
	Aroclor 1254	5	240 ± 14[c]	1.78 ± 0.14[c]
56	Control	5	155 ± 9[b,c]	1.05 ± 0.02[b]
	Control-starved	5	123 ± 7[b]	1.24 ± 0.06[b]
	Aroclor 1254	5	180 ± 26[c]	1.56 ± 0.18[c]
	Aroclor 1254-starved	5	170 ± 5[c]	2.27 ± 0.05[d]
71	Control	5	163 ± 16[b]	1.04 ± 0.08[b]
	Aroclor 1254	2	227[b]	3.75[c]
106	Control	5	144 ± 7[b]	0.89 ± 0.07[b]
	Control-starved	5	157 ± 6[b]	0.91 ± 0.07[b]
	Aroclor 1254	5	174 ± 9[b]	1.13 ± 0.12[b]
	Aroclor 1254-starved	5	163 ± 14[b]	1.68 ± 0.20[c]
120	Control	5	111 ± 3[b]	0.82 ± 0.04[b]
	Control-starved	5	94 ± 8[b]	0.79 ± 0.02[b]
	Aroclor 1254	5	140 ± 10[c]	0.91 ± 0.02[b,c]
	Aroclor 1254-starved	5	137 ± 3[c]	1.15 ± 0.07[c]
148	Control	5	96 ± 8[b]	0.69 ± 0.03[b]
	Control-starved	5	101 ± 7[b]	0.84 ± 0.03[c]
	Aroclor 1254	5	138 ± 16[b]	0.91 ± 0.04[c,d]
	Aroclor 1254-starved	5	119 ± 21[b]	1.00 ± 0.04[d]

[a]Groups of 5 rats were administered corn oil or Aroclor 1254, 10 mg/kg/day for 14 days po. In some cases, animals were starved for 4 days prior to sacrifice.

[b-d]Values with same superscript are not significantly different from one another (P>0.05).

TABLE 26. EFFECT OF HALOGENATED BENZENES ON ACETANILIDE HYDROXYLASE AND ACETANILIDE ESTERASE

Treatment[a]	N	Acetanilide hydroxylase, (nmols/mg protein/min)	N	Acetanilide esterase, (nmols/mg protein/min)
Corn oil	6	2.61 ± 0.17	5	14.8 ± 3.1
1,2,4-Trichlorobenzene	6	3.20 ± 0.23	6	31.5 ± 2.1[b]
1,3,5-Trichlorobenzene	6	2.36 ± 0.15	6	29.1 ± 9.6
Hexachlorobenzene	6	3.94 ± 0.32[b]	6	30.0 ± 5.2[b]
Corn oil	6	1.30 ± 0.06	5	6.1 ± 0.8
1,2,4-Tribromobenzene	6	1.31 ± 0.17	6	10.4 ± 1.1[b]
1,3,5-Tribromobenzene	6	2.30 ± 0.23[b]	6	14.8 ± 1.8[b]
Hexabromobenzene	6	4.89 ± 0.39[b]	6	4.7 ± 1.3

[a]0.1 mmol/kg/day for 14 days po.

[b]Significantly different from control ($P<0.05$).

TABLE 27. EFFECT OF HALOGENATED BENZENES ON PROCAINE ESTERASE

Treatment[a]	N	Procaine hydrolysis (µmols/g/hr)
Control	5	3.73 ± 0.21
1,2,4-Trichlorobenzene	5	6.70 ± 0.43[b]
1,3,5-Trichlorobenzene	5	6.61 ± 0.22[b]
Hexachlorobenzene	5	4.91 ± 0.48[b]
Control	5	3.58 ± 0.64
1,2,4-Tribromobenzene	4	5.97 ± 0.51[b]
1,3,5-Tribromobenzene	3	5.33 ± 0.61
Hexabromobenzene	5	4.71 ± 0.90

[a]1.0 mmol/kg/day for 14 days po.

[b]Significantly different from control ($P<0.05$).

TABLE 28. EFFECT OF BROMINATED AND CHLORINATED BENZENES ON THE LETHALITY OF MALATHION

Treatment[a]	LD_{50}[c] (mg/kg)	95% Confidence limits
Control	1370	(1356 - 1384)
1,4-Dichlorobenzene	1404	(1360 - 1431)
1,2,4-Trichlorobenzene	1628	(1529 - 1804)
Hexachlorobenzene[b]	1529	(1319 - 1456)
1,4-Dibromobenzene	1408	(1385 - 1422)
1,2,4-Tribromobenzene	2035	(1984 - 2130)
Hexabromobenzene	1434	(1405 - 1476)

[a]Halogenated benzene was administered at a dose of 1 mmol/kg daily for 7 days po to male mice. Controls received corn oil.

[b]0.1 mmol/kg/day.

[c]Malathion was administered on day 8 po and lethality determined 24 hours later.

TABLE 29. EFFECT OF 1,2,4-TRICHLOROBENZENE AND 1,2,4-TRIBROMOBENZENE ON THE LETHALITY OF MALAOXON

Treatment[a]	LD_{50}[b] (mg/kg)	95% Confidence limits
Control	167	(150 - 192)
1,2,4-Trichlorobenzene	337	(263 - 439)
1,2,4-Tribromobenzene	381	(311 - 1182)

[a]Halogenated benzene was administered at a dose of 1 mmol/kg daily for 7 days po to male mice. Controls received corn oil.

[b]Malaoxon was administered on day 8 po and lethality determined 24 hours later.

TABLE 30. EFFECT OF BROMINATED AND CHLORINATED BENZENES ON THE LETHALITY OF PARATHION

Treatment[a]	LD_{50}[c] (mg/kg)	95% Confidence limits
Control	12.1	(10.1 - 13.6)
1,4-Dichlorobenzene	18.1	(16.5 - 21.1)
1,2,4-Trichlorobenzene	51.2	(36.3 - 66.5)
Hexachlorobenzene[b]	25.3	(21.4 - 26.3)
1,4-Dibromobenzene	34.3	(27.0 - 44.0)
1,2,4-Tribromobenzene	69.6	(56.9 - 77.5)
Hexabromobenzene	17.4	(13.0 - 22.0)

[a]Halogenated benzene was administered at a dose of 1 mmol/kg daily for 7 days po to male mice. Controls received corn oil.

[b]0.1 mmol/kg/day.

[c]Parathion was administered on day 8 po and lethality determined 24 hours later.

TABLE 31. EFFECT OF BROMINATED AND CHLORINATED BENZENES ON THE LETHALITY OF PARAOXON

Treatment[a]	LD_{50}[c] (mg/kg)	95% Confidence limits
Control	11.5	(10.9 - 12.0)
1,4-Dichlorobenzene	15.0	(9.8 - 20.9)
1,2,4-Trichlorobenzene	37.8	(33.7 - 41.9)
Hexachlorobenzene[b]	19.6	(17.7 - 21.3)
1,4-Dibromobenzene	33.4	(27.1 - 39.7)
1,2,4-Tribromobenzene	54.5	(42.5 - 68.4)
Hexabromobenzene	19.0	(15.3 - 23.5)

[a]Halogenated benzene was administered at a dose of 1 mmol/kg daily for 7 days po to male mice. Controls received corn oil.

[b]0.1 mmol/kg/day.

[c]Paraoxon was administered on day 8 po and lethality determined 24 hours later.

TABLE 32. EFFECT OF 1,2,4-TRICHLOROBENZENE ON CHOLINESTERASE ACTIVITY IN THE BRAIN, LIVER, PLASMA, AND RED BLOOD CELLS OF MICE CHALLENGED WITH MALATHION

Treatment[a]	µmols Substrate[c] hydrolyzed per			
	g brain/min	g liver/min	ml plasma/min	ml RBCs/min
Control	12.72 ± 0.70[d]	6.30 ± 0.39[d]	2.52 ± 0.15[d]	1.27 ± 0.08[d]
Control + Malathion[b]	8.60 ± 0.91[e]	2.45 ± 0.32[e]	1.33 ± 0.09[e]	0.68 ± 0.05[e]
1,2,4-Trichlorobenzene	10.76 ± 1.59[d,e]	5.20 ± 0.43[f]	2.58 ± 0.11[d]	1.96 ± 0.31[f]
1,2,4-Trichlorobenzene + Malathion[b]	11.98 ± 0.27[d]	1.64 ± 0.17[e]	1.23 ± 0.14[e]	1.01 ± 0.12[d,e]

[a]Groups of 5 to 6 adult male mice were administered 1 mmol/kg/day of 1,2,4-trichlorobenzene daily for 7 days po. Controls received corn oil. Value is mean ± S.E.

[b]Malathion was administered 1 g/kg 1 to 2 hours before sacrifice on day 8 po.

[c]Substrate was 74.93 mM acetylthiocholine iodide.

[d-f]Within each tissue, values with the same superscript are not significantly different from one another (P>0.05).

TABLE 33. EFFECT OF 1,2,4-TRIBROMOBENZENE ON CHOLINESTERASE ACTIVITY IN THE BRAIN, LIVER, PLASMA, AND RED BLOOD CELLS OF MICE CHALLENGED WITH MALATHION

Treatment[a]	µmols Substrate[c] hydrolyzed per			
	g brain/min	g liver/min	ml plasma/min	ml RBCs/min
Control	9.75 ± 0.75[d,e]	5.57 ± 0.34[d]	2.39 ± 0.16[d]	1.71 ± 0.38[d,e]
Control + Malathion[b]	4.61 ± 0.59[f]	1.75 ± 0.23[e]	1.20 ± 0.15[e]	0.47 ± 0.08[f]
1,2,4-Tribromobenzene	8.46 ± 0.42[d]	5.22 ± 0.55[d]	2.30 ± 0.21[d]	2.08 ± 0.36[e]
1,2,4-Tribromobenzene + Malathion[b]	10.50 ± 0.31[e]	1.42 ± 0.18[e]	1.19 ± 0.12[e]	1.25 ± 0.21[d]

[a]Groups of 5 to 6 adult male mice were administered 1 mmol/kg/day of 1,2,4-tribromobenzene daily for 7 days po. Controls received corn oil. Value is mean ± S.E.

[b]Malathion was administered 1 g/kg 1 to 2 hours before sacrifice on day 8 po.

[c]Substrate was 74.93 mM acetylthiocholine iodide.

[d-f]Within each tissue, values with the same superscript are not significantly different from one another (P>0.05).

TABLE 34. EFFECT OF BROMINATED AND CHLORINATED BENZENES ON MALATHION CARBOXYLESTERASE

Treatment[a]	µmols Malathion hydrolyzed per g liver/min	µmols Malathion hydrolyzed per ml plasma/min
Control	3.81 ± 0.39[b]	4.81 ± 0.45[b]
1,4-Dichlorobenzene	7.43 ± 0.78[c,d]	6.07 ± 0.47[b,c]
1,2,4-Trichlorobenzene	6.18 ± 0.47[c]	7.62 ± 0.88[c]
Hexachlorobenzene	6.71 ± 0.77[c,d]	7.35 ± 0.30[c]
1,4-Dibromobenzene	8.89 ± 1.15[d,e]	6.18 ± 0.49[b,c]
1,2,4-Tribromobenzene	10.47 ± 0.76[e]	9.09 ± 0.38[e]
Hexabromobenzene	6.60 ± 0.76[c,d]	5.79 ± 0.38[b]

[a]Groups of 5 to 6 adult male mice were administered 1 mmol/kg of halogenated benzene (0.1 mmol/kg for hexachlorobenzene) daily for 7 days po. Controls received corn oil. Value is mean ± S.E.

[b-e]Within each tissue, values with the same superscript are not significantly different from one another (P>0.05).

TABLE 35. EFFECT OF BROMINATED AND CHLORINATED BENZENES ON MALAOXON CARBOXYLESTERASE

Treatment[a]	µmols Malaoxon hydrolyzed per g liver/min	ml plasma/min
Control	7.23 ± 0.67[b,c]	3.45 ± 0.21[b]
1,4-Dichlorobenzene	7.71 ± 0.69[b,c]	3.21 ± 0.11[b]
1,2,4-Trichlorobenzene	8.46 ± 0.32[c]	4.92 ± 0.33[c,d]
Hexachlorobenzene	6.47 ± 0.57[b]	3.40 ± 0.17[b]
1,4-Dibromobenzene	6.52 ± 0.39[b]	3.67 ± 0.16[b,c]
1,2,4-Tribromobenzene	7.93 ± 0.41[b,c]	6.17 ± 0.25[d]
Hexabromobenzene	4.01 ± 0.16[d]	3.07 ± 0.12[b]

[a]Groups of 5 to 6 adult male mice were administered 1 mmol/kg of halogenated benzene (0.1 mmol/kg for hexachlorobenzene) daily for 7 days po. Controls received corn oil. Value is mean ± S.E.

[b-d]Within each tissue, values with the same superscript are not significantly different from one another (P>0.05).

TABLE 36. EFFECT OF 1,2,4-TRICHLOROBENZENE ON CHOLINESTERASE ACTIVITY IN THE BRAIN, LIVER, PLASMA, AND RED BLOOD CELLS OF MICE CHALLENGED WITH PARAOXON

Treatment[a]	µmols Substrate[c] hydrolyzed per			
	g brain/min	g liver/min	ml plasma/min	ml RBCs/min
Control	9.27 ± 0.79[d]	5.37 ± 0.64[d]	3.24 ± 0.52[d]	0.87 ± 0.10[d]
Control + Paraoxon[b]	2.42 ± 2.19[e]	0.75 ± 0.21[e]	0.19 ± 0.06[e]	0.02 ± 0.01[e]
1,2,4-Trichlorobenzene	10.01 ± 0.90[d]	3.86 ± 0.65[f]	3.07 ± 0.29[d]	1.64 ± 0.34[f]
1,2,4-Trichlorobenzene + Paraoxon[b]	8.05 ± 1.74[d]	2.26 ± 0.47[g]	0.19 ± 0.05[e]	0.04 ± 0.02[e]

[a]Groups of 4 to 5 adult male mice were administered 1 mmol/kg/day of 1,2,4-trichlorobenzene daily for 7 days po. Controls received corn oil. Value is mean ± S.E.

[b]Paraoxon was administered 10 mg/kg 1 to 2 hours before sacrifice on day 8 po.

[c]Substrate was 74.93 mM acetylthiocholine iodide.

[d]Within each tissue, values with the same superscript are not significantly different from one another (P>0.05).

TABLE 37. EFFECT OF 1,2,4-TRIBROMOBENZENE ON CHOLINESTERASE ACTIVITY IN THE BRAIN, LIVER, PLASMA, AND RED BLOOD CELLS OF MICE CHALLENGED WITH PARAOXON

Treatment[a]	µmols Substrate[c] hydrolyzed per			
	g brain/min	g liver/min	ml plasma/min	ml RBCs/min
Control	10.09 ± 0.63[d]	2.91 ± 0.27[d]	2.90 ± 0.53[d]	2.07 ± 0.47[d]
Control + Paraoxon[b]	1.57 ± 0.43[e]	0.51 ± 0.06[e]	0.27 ± 0.03[e]	0.11 ± 0.01[e]
1,2,4-Tribromobenzene	8.57 ± 0.59[d]	2.55 ± 0.16[d]	3.38 ± 0.29[d]	2.16 ± 0.46[d]
1,2,4-Tribromobenzene + Paraoxon[b]	7.82 ± 1.00[d]	1.63 ± 0.34[f]	0.29 ± 0.05[e]	0.09 ± 0.03[e]

[a]Groups of 4 to 5 adult male mice were administered 1 mmol/kg/day of 1,2,4-tribromobenzene daily for 7 days po. Controls received corn oil. Value is mean ± S.E.

[b]Paraoxon was administered 10 mg/kg 1 to 2 hours before sacrifice on day 8 po.

[c]Substrate was 74.93 mM acetylthiocholine iodide.

[d-f]Within each tissue, values with the same superscript are not significantly different from one another (P>0.05).

TABLE 38. EFFECT OF BROMINATED AND CHLORINATED BENZENES ON PARAOXON DEARYLATION BY MOUSE PLASMA AND LIVER

Treatment[a]	Rate of Dearylation[b]			
			Microsomal	
	Plasma	105,000 x g Supernatant	Mixed function[c] oxidase	Esterase[d]
Control	14.20 ± 1.50[e,f]	0[e]	1.09 ± 0.41[e]	3.96 ± 0.53[e]
1,4-Dichlorobenzene	15.01 ± 2.13[f]	0[e]	3.26 ± 0.49[f]	4.13 ± 0.42[e]
1,2,4-Trichlorobenzene	3.25 ± 0.33[g]	0.15 ± 0.03[f]	5.41 ± 0.87[g]	6.38 ± 0.86[f]
Hexachlorobenzene	11.89 ± 1.93[e,f]	0.09 ± 0.02[f,g]	3.47 ± 0.88[f,g]	7.19 ± 0.60[f]
1,4-Dibromobenzene	9.00 ± 3.02[e]	0.03 ± 0.03[e,g]	2.14 ± 0.22[e,f]	1.57 ± 0.40[g]
1,2,4-Tribromobenzene	0[g]	0.09 ± 0.02[f,g]	8.70 ± 0.68[h]	0.87 ± 0.21[g]
Hexabromobenzene	12.91 ± 1.80[e,f]	0.11 ± 0.02[f]	1.76 ± 0.31[e,f]	7.25 ± 0.64[f]

[a]Groups of 5 adult male mice were administered 1 mmol/kg/day of halogenated benzene (0.1 mmol/kg/day for hexachlorobenzene) daily for 7 days po. Controls received corn oil.

[b]nmols p-Nitrophenol formed/min/ml plasma or mg protein. Value is mean ± S.E.

[c]Contains 1.2 mM NADPH and 0.1 mM EDTA as inhibitor of the esterase.

[d]Contains no NADPH or EDTA.

[e-h]Within each tissue, values with same superscript are not significantly different from one another (P>0.05).

TABLE 39. EFFECT OF HALOGENATED BENZENES ON ARYLESTERASE[a]

Treatment[b]	Serum µmols/min/ml	Liver µmols/min/g
Corn Oil	129 ± 1[c]	126 ± 2[c]
1,2,4-Trichlorobenzene	99 ± 6[d]	179 ± 9[d]
1,3,5-Trichlorobenzene	137 ± 6[c]	148 ± 11[c]
Hexachlorobenzene	82 ± 8[d]	189 ± 4[d]
Corn Oil	134 ± 7[c]	153 ± 3[c]
1,2,4-Tribromobenzene	147 ± 14[c]	206 ± 4[d]
1,3,5-Tribromobenzene	98 ± 12[d]	171 ± 5[e]
Hexabromobenzene	134 ± 7[c]	165 ± 5[c,e]

[a]Phenyl acetate as substrate.

[b]0.1 mmols/kg/day for 14 days po to groups of 5 rats.

[c-e]Values with same superscript are not significantly different from one another (P>0.05).

REFERENCES

Akao, T. and Omura, T. (1972). Acetanilide-hydrolyzing esterase of rat liver microsomes. 1. Solubilization, purification and intramicrosomal localization. J. Biochem. 72, 1245-1256.

Arrhenius, E., Renberg, L., Johansson, L. and Zetterqvist, M.A. (1977). Disturbance of microsomal detoxication mechanisms in liver by chlorophenol pesticides. Chem-Biol. Interactions 18, 35-46.

Bauer, J.D. Hemoglobin, in S. Frankel, S. Reitman and A.C. Sonnenwirth (eds.), Gradwohl's Clinical Laboratory Methods and Diagnosis, C.V. Mosby Co., St. Louis, 1970, p. 396.

Cammer, P.A. and Hollingworth, R.M. (1976). Unusual substrate specificity in the oxidative dearylation of paraoxon analogs by mouse hepatic microsomal enzymes. Biochem. Pharmacol. 25, 1799-1807.

Carlson, G.P. (1977). Chlorinated benzene induction of hepatic porphyria. Experientia 33, 1627-1629.

Carlson, G.P. and Tardiff, R.G. (1976). Effect of chlorinated benzenes on the metabolism of foreign organic compounds. Toxicol. Appl. Pharmacol. 36, 383-394.

Carlson, G.P. and Tardiff, R.G. (1977). Effect of 1,4-dibromobenzene and 1,2,4-tribromobenzene on xenobiotic metabolism. Toxicol. Appl. Pharmacol. 42, 189-196.

Cohen, S.D., Ehrich, M. and Orzech, D. (1977). Manometric and spectrophotometric procedures for measurement of procaine hydrolysis by mouse liver in vitro. Analyt. Biochem. 80, 168-175.

Dallner, G. (1963). Studies on the structural and enzymatic organization of the membranous elements of liver microsomes. Acta Pathol. Microbiol. Scand. (Suppl.) 166, 7-41.

Doss, V.M. and Schmidt, A. (1971). Quantitative bestimmung von δ-aminolavulinsaure und porphobilinogen im urin mit ionenaustauschchromatographic-fertig saulen. Z. Klin. Chem. Biochem. 9, 99-102.

Ellman, G., Courtney, D., Andres, V. and Featherstone, R. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95.

Finney, D.J. Probit Analysis, Cambridge Press, London, 1971, p. 50.

Gerlach, U. Sorbitol Dehydrogenase, in H.U. Bergmeyer (ed.), Methods of Enzymatic Analysis, Academic Press, New York, 1965, p. 761.

Goldstein, J.A., Hickman, P. and Jue, D.L. (1974). Experimental porphyria induced by polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 27, 437-448.

Harper, A.E. Glucose-6-phosphatase, in H.U. Bergmeyer (ed.), Methods of Enzymatic Analysis, Academic Press, New York, 1965, p. 788.

Johnstone, G.J., Ecobichon, D.J. and Hutzinger, O. (1974). The influence of pure polychlorinated biphenyl compounds on hepatic function in the rat. Toxicol. Appl. Pharmacol. 28, 66-81.

Kinoshita, F.K., Frawley, J.P. and DuBois, K.P. (1966). Quantitative measurement of induction of hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rats. Toxicol. Appl. Pharmacol. 9, 505-513.

Krisch, K. and Staudinger, Hj. (1961). Untersuchungen zur enzymatischen hydroxylierung. Hydroxylierung von acetanilid und deren beziehungen zur mikrosomalen pyridinnucleotidoxydation. Biochem. Z. 334, 312-327.

Lambert, G. and Brodeur, J. (1976). Influence of starvation and hepatic microsomal enzyme induction on the mobilization of DDT residues in rats. Toxicol. Appl. Pharmacol. 36, 111-120.

Lorentz, K., Flatter, B. and Augustin, E. (1979). Arylesterase in serum: Elaboration and clinical application of a fixed-incubation method. Clin. Chem. 25, 1714-1720.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Lucier, G.W., McDaniel, O.S. and Matthews, H.B. (1971). Microsomal rat liver UDP glucuronyl-transferase: Effects of piperonyl butoxide and other factors on enzymic activity. Arch. Biochem. Biophys. 145, 520-530.

Mauzerall, D. and Granick, S. (1956). The occurrence and determination of δ-aminolevulinic acid and porphobilinogen in urine. J. Biol. Chem. 219, 435-446.

Nebert, D.W. and Gelboin, H.V. (1968). Substrate inducible microsomal aryl hydroxylase in mammalian cell culture. 1. Assay and properties of induced enzyme. J. Biol. Chem. 243, 6242-6249.

Piper, W.N., Condie, L.W. and Tephly, T.R. (1973). The role of substrates for glycine acyltransferase in the reversal of chemically induced porphyria in the rat. Arch. Biochem. Biophys. 159, 671-677.

Steel, R.G.D. and Torrie, J.H. Principles and Procedures of Statistics, McGraw-Hill, New York, 1960, p. 107.

Talcott, R.C. (1979). Hepatic and extrahepatic malathion carboxylesterase assay and localization in rat. Toxicol. Appl. Pharmacol. 47, 145-150.

Villeneuve, D.C., Phillips, W.E.J. and Syrotiuk, J. (1970). Modification of microsomal enzyme activity and parathion toxicity in rats. Bull. Environ. Contam. Toxicol. 5, 125-132.